ABSTRACT

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A STUDY OF MITOTIC RECOMBINATION IN YEAST  
BY PEDIGREE ANALYSIS

Pedigree analysis proved useful to study U.V. induced mitotic recombination in Saccharomyces. Twenty-two recombinant chromosome arms were analysed. Eighteen showed reciprocal segregations in the first post-irradiation generation. The other four pedigrees displayed a generation delay between the cross-over event and the marker segregation. To account for delayed segregation, the exchange of "long" regions of single-stranded DNA was proposed. A model was suggested which accounts for reciprocal and non-reciprocal recombination by different mechanisms.

Lethal sectoring was examined in haploid yeast with altered radiation sensitivities. Only an X-ray sensitive, recombinationless strain showed greater than normal spontaneous lethal sectoring. X-rays did not induce lethal sectoring in haploids. U.V. induction of lethal sectoring in a U.V. sensitive and "wild type" strain were compared, it was found that the U.V. sensitive strain showed significantly greater lethal sectoring than the "wild type". The influence of lethal sectoring upon recombination and mutation data was considered.
PEDIGREE ANALYSIS OF MITOTIC RECOMBINATION IN YEAST

by

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# TABLE OF CONTENTS

## INTRODUCTION

### LITERATURE REVIEW

- Induced Mitotic Recombination
- The Mechanism of Recombination
- The Hybrid DNA Models
- DNA Repair After U.V. Irradiation
- Pedigree Analysis and Recombination
- Pedigree Analysis and Lethal Sectoring

### MATERIALS AND METHODS

- Strains
- Media
- Treatments
- Pedigree Analysis
- Genetic Analysis

### RESULTS

1. A Study of Mitotic Recombination by Pedigree Analysis
   - Preliminary Experiments
   - The Study of U.V. Induced Mitotic Recombination by Pedigree Analysis

2. Lethal Sectoring in Haploid Strains With Altered Radiation Sensitivity
   - Survival Curves
   - Spontaneous Lethal Sectoring
INTRODUCTION

The experiments reported in this study were undertaken for the following purposes: (I) to examine ultra-violet (U.V.) induced mitotic recombination in *Saccharomyces cerevisiae*, using pedigree analysis to determine a) the number of generations after U.V. irradiation over which induced mitotic recombination continues to occur and b) whether the segregation of genetic markers can occur a generation removed from the cross-over event; (II) to determine the relationship between radiation sensitivity and lethal sectoring.

I. Classically, mitotic recombination has been assumed to involve whole-chromatid exchanges (Stern 1936). A consequence of this model is that both the mitotic cross-over event and segregation of the involved genetic markers occur in the same division. On the other hand, half-chromatid (i.e. single-stranded DNA) exchanges would result in the segregation of the involved genetic markers one generation removed from the cross-over event. This study will determine whether such delayed segregations do occur.

The occurrence of delayed segregations would provide evidence concerning the presently favoured "hybrid-repair" models of recombination which have been proposed by Holliday (1964) and by Hastings and Whitehouse (1964). These models depend upon the formation of short regions of hybrid DNA. The correction of heterologous or "mismatched" DNA is thought to
account for gene conversion. According to Whitehouse (1965b), 5:3 or 3:5 gene ratios, or half-chromatid conversions, are the result of the partial correction of heterologous DNA. Taking these ratios as evidence for partially corrected "mismatched" DNA, it is likely that uncorrected heterologous DNA also exists. It was believed that the segregation of u.v. corrected "mismatched" DNA would be detectable by pedigree analysis and if detected would provide further evidence for the involvement of hybrid DNA in recombination.

There are conflicting reports concerning the number of post-irradiation generations over which recombination is induced by U.V. Holliday (1962) has reported that U.V. induced mitotic recombination occurs entirely in the first post-irradiation generation, while Haefner (1966) has reported that induced recombination continues for several post-irradiation generations. Wood (1967) has demonstrated that in Aspergillus nidulans, segregations can occur in at least two post-irradiation generations.

II. Pedigree analysis often uncovers a particular event—one of the pedigree products is lost as a "permanent" line of descent and fails to produce a macroscopic colony. This is the phenomenon of lethal sectoring. After much investigation, the mechanism of lethal sectoring remains unknown (for review see James 1967). There is evidence however, that repair and lethal sectoring may be related. The reasons are as follows: the greater the time between X-ray irradiation and the first
division, the fewer the lethal sectors, suggesting that the longer time before the first division allows more time for repair of damage which would have resulted in lethal sectors (James and Werner 1966). Furthermore, mercaptoethanol treatment which delays mitosis, probably allowing more time for repair, reduces lethal sectoring (James and Werner in press). The following sequence of events has been suggested (James and Werner 1966): partial repair of lethal damage resulting in lethal sectors; further repair (or repair of damage which would have produced lethal sectors) resulting in cells with full colony forming ability.

Furthermore, Nasim and Saunders (1968) have reported that two different U.V. sensitive strains of Schizosaccharomyces pombe demonstrate a significantly greater amount of spontaneous lethal sectoring than normal and Haefner (1968) has reported that rec- strains of E. coli demonstrate increased lethal sectoring. This strongly suggests that lethal sectoring and radiation sensitivity are related. Using some of the available strains of yeast which demonstrate altered radiation sensitivity (for review see Resnick 1968), the study of the relationship between repair and lethal sectoring was pursued.
Recombination is a process of major biological importance. The classic definition (King 1968) includes the rearrangement of genetic material either by crossing-over, i.e., the exchange of parts of homologous chromosomes, or by independent chromosome assortment. Contemporary usage of the term stresses the former event. Recent evidence suggests that mitotic recombination is related to repair and mutation (Witkin 1968).

Mitotic recombination was first demonstrated in Drosophila by Stern (1936). Proof of mitotic crossing-over depends upon the recovery of the reciprocal products which would be expected of such a process. In fungi, such evidence was obtained by Roper and Pritchard (1955) in a case of spontaneous intragenic recombination within the ad₅ loci of *Aspergillus nidulans*. In the single case which they found, both recombinant strands had segregated into one nucleus (Figure 1A). Käfer (1961), in an extensive analysis of spontaneous mitotic recombination, isolated the reciprocal products of crossing-over in four cases. In one-half the cases she found that the recombinant chromosomes had segregated into one nucleus; in the other half they had segregated such that one parental, and one recombinant chromosome had segregated into each of the product nuclei (Figure 1B). In the latter case, the mitotic products differ from each other as well as from the parental type and are referred to as twin spots.
Studies of mitotic recombination in fungal organisms are well documented and have been most valuable, but the occurrence of this phenomenon in higher organisms is uncertain, although various cases have been postulated from studies on mice (see review by Grunberg 1966). Smithes (1963) and Edelman and Gally (1967) have suggested that human antibody variability is due to unequal mitotic crossing-over. The lack of reports of recombination in tissue culture systems has, however, cast further doubt upon its occurrence in higher organisms.

**Induced Mitotic Recombination**

Mitotic crossing-over is a rare event. In Aspergillus it
occurs one to five times per hundred mitoses (Pontecorvo 1956; Käfer 1961). It is also an infrequent event in *Ustilago maydis* (Holliday 1961). In *Saccharomyces cerevisiae*, the frequency of mitotic recombination between linked genes is about 10% of the meiotic value which is large when compared to the value of 0.1% given for *Aspergillus* (Casselton 1965).

Fortunately, the frequency of mitotic recombination can be increased by ultra-violet (U.V.) irradiation. This was first demonstrated by James and Lee-Whiting (1955) for intergenic crossing-over in yeast. Ultra-violet irradiation has been shown to induce mitotic recombination in *Penicillium chrysogenum* (Morpurgo and Sermonti 1959), in germinating *Aspergillus* conidia (Käfer and Chen 1964; Wood and Kafer 1967) and in *Ustilago maydis* (Holliday 1961). X-rays have also found to induce mitotic crossing-over (Morpurgo 1962) while Käfer (1963) has obtained similar results with Cobalt 60 gamma irradiation of *Aspergillus*. Fogel and Hurst (1963) have demonstrated a direct dependence of recombination frequencies in yeast upon U.V. dosage. Manney and Mortimer (1964) have found that the X-ray induction of mitotic recombination in yeast is also dose dependent.

Chemical agents also induce mitotic crossing-over. For example: Mitotic recombination is stimulated by formaldehyde in *Aspergillus* (Fratello, Morpurgo and Sermonti 1960), mitomycin C induces mitotic recombination in *Ustilago* and
Saccharomyces (Holliday 1964) as well as in Aspergillus (Shanfield unpublished) and fluordeoxyuridine (FUDR) has been shown to induce mitotic recombination in Aspergillus (Beccari, Modigliani and Morpurgo 1967; Shanfield 1968). Zimmerman and his colleagues (Zimmerman and von Laer 1967; Zimmerman, Schwaier and von Laer 1966) have demonstrated that a large number of agents such as nitrous acid, diethylsulphate, ethyleneimine and the monofunctional and bifunctional alkylating nitrosamides can induce mitotic recombination in *Saccharomyces cerevisiae*.

The Mechanism of Recombination

A number of mechanisms have been proposed to explain recombination (for review see Fincham and Day 1965). Before any model can be accepted, it must explain several anomalies which have been reported in different organisms. These are: (1) non-reciprocal recombination or gene conversion, (2) polarity, (3) negative interference.

Gene Conversion:—In diploid organisms which possess a conventional meiotic cycle and which can be examined by tetrad analysis, one expects that the alleles of a heterozygous strain will segregate in the ratio of two "wild type" to two mutant spores. However, in 1955 Mitchell published data which showed that the pyridoxine (pdx) independent recombinants from crosses of Neurospora pdx-mutants did not necessarily arise from reciprocal recombination. Thus, some asci showed a 3:1 ratio
which would have been expected if one of the four strands had been converted from mutant to "wild type" at the first meiotic prophase. Abnormal tetrad segregation had been reported earlier in *Funaria hygrometrica* (von Wettstein 1924), *Coprinus fumetarius* (Brunswick 1929) in the Ascomycetes (Zickler 1934; Wülker 1935) and in yeast (Lindegren 1949; 1953).

Lindegren and his colleagues (1953; 1956) proposed a theory of gene conversion to account for abnormal tetrad ratios. He explained the abnormal segregations as "allele induced mutations" and adopted the term conversion which Winkler (1930) had defined as a mutation of a gene caused by its allele. The theory of gene-conversion proposed by Winkler is known to be invalid in its original form, since it attempted to explain all recombination by gene conversion. The recovery of the reciprocal products of crossing-over in Drosophila by Stern (1936) showed that all recombinations were not conversions. This discovery however, does not preclude gene-conversion from occurring. Today the terms gene conversion, non-reciprocal recombination and heteroallelic repair are used interchangeably to describe abnormal tetrad segregations.

Recombination and Gene Conversion:-There is a great deal of evidence that gene conversion and recombination are related. Information from studies on Neurospora (Mitchell 1955; Freese 1957; Fogel 1964) showed that convertant ascospores have a much higher frequency of reciprocal recombination of outside
markers than non-convertant ascospores. However, it remains uncertain whether gene conversion and recombination are dependent upon the same mechanism or are favoured by the same conditions.

The coincident relationship between conversion and outside marker crossing-over in meiotic products of Neurospora was not confirmed in mitotically dividing yeast (Roman 1956). Furthermore, Roman and Jacob (1958) found that U.V. induced conversion had little effect on crossing-over of outside markers. More recently, however, other investigators (Fogel and Hurst 1963; Hurst and Fogel 1964) demonstrated that reciprocal and non-reciprocal events are often coincident in yeast. They suggest that reciprocal and non-reciprocal mitotic recombination have common precursors for their expression.

Parry and Cox (1968a) examined the effects of U.V. upon synchronously dividing S. cerevisiae. They irradiated samples from a synchronous culture at different times in the cell cycle and compared the radiation effects on cell death, inter- and intragenic recombination. It was found that temporal differences existed between conversion and intergenic recombination. Conversion occurred at a peak about one half hour after the intergenic peak. However, these results are directly contrary to the results of Esposito (1968b) with S. cerevisiae. It is possible that the difference in results is due to differences in the degree of synchrony obtained in each experiment.
Mitchell (1957) found evidence that changes in temperature altered conversion frequencies without a corresponding alteration of crossing-over frequencies.

Parry and Cox (1968b) found that after U.V. treatment, photoreactivation (PHR) and liquid holding recovery (LHR) had different effects upon gene conversion and reciprocal recombination. Photoreactivation had no effect upon crossing-over although it reduced conversion frequencies by as much as a factor of 10. Liquid holding recovery halved the amount crossing-over but greatly increased the amount of conversion.

It can be concluded that if recombination and gene conversion depend upon the same original event, each phenomenon has its own pathway.

Polarity:-Polarity exists when tetrad analysis reveals a preferential direction of non-reciprocal recombination between alleles. Rizet, Lissouba and their colleagues first observed this phenomenon while studying spore colour mutants of *Ascobolus immersus* which can be detected by direct observation (Rizet et al 1960; Lissouba and Rizet 1960; Lissouba et al 1962). They examined about two thousand white spored mutants and produced several series of closely linked markers. Certain markers in the aberrant tetrads occurred in excess. They concluded that conversion showed polarity and increased unidirectionally within short regions of the gene free from reciprocal recom-
The unit of polarity was termed a polaron.

The stringent polarity of the type in Ascobolus was not found by Siddiqui and Putrament (1963) in *Aspergillus nidulans* nor by Stadler and Towe (1963) in Neurospora. In these organisms the polarity seems less severe. This may be due to the fact that more rigorous investigations are possible in Ascobolus because the spores in this organism can be examined visually and do not require dissection. However, in 1964 Rossignal did a very extensive analysis of conversion in Ascobolus and determined that reversals in polarity and the occasional reciprocal exchange occurs within the proposed polaron. More recently, Fogel and Mortimer (1969) reported the complete lack of polarity in intragenic recombination in Saccharomyces.

**Negative Interference:** Pritchard (1955) when studying mitotic intragenic recombination in the Aspergillus ade locus, observed negative interference. Negative interference occurs when rare cross-over events occur simultaneously with a very much greater frequency than expected. This phenomenon was mentioned earlier in the discussion of the relationship between recombination and gene conversion. Negative interference has been found in Neurospora (Freese 1957; Case and Giles 1958a,b), in phage (Chase and Doerman 1958) and in Saccharomyces (Sherman and Roman 1963).

Case and Giles (1958a,b; 1963; 1964) have studied a number of
two and three point crosses in pantothenic acid requiring mutants (pan-2) of *N. crassa*. They found that the multiple events occurred fifty to one hundred times more frequently than expected. The conversion of the end markers in a three point intragenic cross accounted for a large part of the pan-2 prototrophs even though a minimum of four recombination events were required between these adjacent allelic markers.

The Hybrid DNA Models

A number of models of recombination have been proposed to account for recombination, conversion, polarity and negative interference (for review see Fincham and Day 1965; Hastings and Whitehouse 1964; Whitehouse 1965b; Holliday 1964).

The recombination models presently favoured are those of Holliday (1964) and Whitehouse (1965a). They are similar in that they (1) do not require a net synthesis of DNA (2) involve the formation of hybrid DNA and (3) if the region of hybrid DNA contains mismatched pairs, the repair of these regions.

The repair models are diagrammed in Figures 2 and 3. Both require the unwinding of strands in each chromatid, strand breakage and then the rewinding with the complementary strand of the opposing chromatid. A further comparison between the Holliday and the Whitehouse models is shown in Table I.
Figure 2. Whitehouse Model of Crossing-Over and Gene Conversion. See Text for Discussion.
Figure 3. Holliday Model of Crossing-Over and Gene Conversion.
See Text for Discussion.
### Table I. Comparison of Whitehouse's and Holliday's models of recombination.

<table>
<thead>
<tr>
<th>STEP</th>
<th>HOLLIDAY MODEL</th>
<th>WHITEHOUSE MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strands involved</td>
<td>Original</td>
<td>Newly synthesized</td>
</tr>
<tr>
<td>Polarity of rewinding strands</td>
<td>Alike</td>
<td>Different</td>
</tr>
<tr>
<td>Results</td>
<td>After breakage and exchange, yields products with and without crossing-over of outside markers.</td>
<td>After breakage additional DNA synthesis is required and the supernumary strands of DNA must be destroyed. Crossing-over always occurs when model is in the simplest form.</td>
</tr>
<tr>
<td>Crossing-over of outside markers</td>
<td>Both alternatives giving about 50% with crossing-over</td>
<td>Always in simplest form but Whitehouse has hypothesized a &quot;double event&quot; which may remove crossing-over of outside markers.</td>
</tr>
<tr>
<td>Conversion</td>
<td>Intrinsic to mechanism by repair of hybrid DNA</td>
<td>Same as Holliday's model</td>
</tr>
</tbody>
</table>
The advantage of these recombination models is that they completely explain the present data without requiring further hypothesis. They predict crossing-over equally well in any of the four double strands (assuming that one chromatid represents one DNA molecule). Thus three and four stranded double crossing-over can be explained. The repair models also explain the 3:1 ratios of conversion and the more complicated 5:3 ratios caused by half-chromatid conversions found in Sordaria (Kitani, Olive and El Ani 1961; 1962), in Ascobolus (Lissouba, Mousseau, Rizet and Rossignol 1962) and in Neurospora (Case and Giles 1964).

The recombination models of both Whitehouse and Holliday have been termed "hybrid-repair" models as they depend upon the repair of heterologous hybrid DNA. Since U.V., X-rays and a variety of mutagenic agents increase recombination, it becomes necessary to examine what repair mechanisms are available to the cell, and how these mechanisms are related to recombination.

DNA Repair After U.V. Irradiation

Although damage to cellular components other than DNA could result in killing, these effects are considered insignificant when compared with the DNA damage (Haynes 1966). The exposure of cells to U.V. results in the formation of pyrimidine dimers in the DNA (Wacker, Dellweg and Jacherts 1962). Thymine dimers are most common but cytosine-thymine and cytosine-
cytosine dimers are also produced (Wacker 1963; Smith 1963; Setlow and Carrier 1966; Setlow, Carrier and Bollum 1965a,b).

Two kinds of repair are known whereby these dimers are removed from DNA (1) photoreactivation and (2) excision or "dark" repair. Photoreactivation was first demonstrated by Kelner (1949) and it has been found that pyrimidine dimers are split in situ by a light-dependent enzyme (Wulff and Rupert 1962; Setlow and Setlow 1963; Cook 1967). This enzyme has been isolated from yeast (Rupert 1960; Muhammed 1966) and its kinetics have been analysed (see review by Rupert and Harm 1966). Dark repair, on the other hand, appears to be much more complicated than photoreactivation and apparently involves a series of enzymatically controlled steps.

The discovery of bacterial strains which are resistant to U.V. irradiation provided the first evidence for dark repair (Setlow et al 1963; 1964; Bollum and Setlow 1963). Repair mechanisms involving the removal of induced thymine dimers were suggested by Setlow and Carrier (1964) and by Boyce and Howard-Flanders (1964).

Setlow and Carrier (1964) demonstrated that the release of dimers from the DNA into free TCA-soluble components resulted in concomitant recovery of biological activity of the bacterial DNA. They postulated the excision of the dimer and its replacement by a non-conservative repair synthesis utilizing the still intact complementary strand. Evidence for this "repair replication" came from Pettijohn and Hanawalt (1964). In their
experiments, control and U.V.-irradiated thymine requiring
E. coli cultures were grown in the absence of thymine but in
the presence of the heavy thymine analogue 5-bromouracil (5-BU).
Cesium-chloride density-gradient analysis of the DNA revealed
that the 5-BU had been incorporated into very short segments of
the DNA. These regions of "non-conservative" DNA replication
were randomly distributed through the genome.

It is not known whether excision proceeds the repair or
is coincident with it. Howard-Flanders and Boyce (1966) suggest
that excision proceeds repair replication while Haynes (1966)
supports the coincident mechanism. The models are diagrammed in
Figure 4.

\[\text{Figure 4. A comparison of "Cut and Patch" (upper) and "Patch}
\text{and Cut" (lower).}\]
"Repair replication" is also known to correct damage from Mitomycin C (Boyce and Howard-Flanders 1964), nitrogen mustard (Hanawalt and Haynes 1965) and N-methyl-N-nitro-N-nitrosoguanidine (Cerda-Olmeda and Hanawalt 1967). Boyce and Howard-Flanders (1964) suggested that the enzymes involved in dark repair recognize distortions in the phosphodiester back-bone of the DNA. Abnormal base pairing could result in considerable distortion of the double-helix which would result in small single strand loops (Lehman 1967). An enzyme eg. endonucleases which were specific for single strands would recognize these distortions and initiate repair. (Linn and Lehman 1965 and Healy et al 1963).

From the hybrid DNA recombination models of Whitehouse and Holliday described earlier it follows that recombination and dark repair are possibly related in their enzymatic requirements of recognition and incision steps. In fact, strains which are radiation sensitive and recombination-deficient have been isolated in a number of micro-organisms, such as E. coli (Clark and Margulies 1965; Howard-Flanders and Boyce 1966; and Howard-Flanders and Theriot 1966), Ustilago (Holliday 1967) Pseudomonas (Holloway 1966) and Saccharomyces (Nakai and Matsumoto 1967).

Post-Replication Repair:-The removal of thymine dimers from the damaged DNA may not be a necessary prerequisite to DNA repair. The suggestion that some strains of E. coli can replicate their DNA and form colonies despite the presence of unrepaired
pyrimidine dimers in their DNA was made by Witkin (1966). This was substantiated by Bridges and Munson (1968) who found that dimers could remain in the DNA of excision defective strains for several post-irradiation generations. Recently, Howard-Flanders, Rupp and Wilkins (1968) have demonstrated that daughter strands of \textit{E. coli} K-12 containing unexcised pyrimidine dimers, are discontinuous. They hypothesize that the thymine dimers block DNA replication, producing gaps in the newly replicated strands. If the daughter strands are examined an hour after replication rather than immediately after replication, the discontinuities disappeared. Rupp and Howard-Flanders (1968) have termed this "post-replication" repair. This repair does not act upon the dimers themselves, but rather upon the gaps. Witkin (1968) has suggested that these gaps, which must be missing at least one nucleotide or they would have been sealed by ligase, be regarded as "secondary lesions".

Rupp and Howard-Flanders (1968) have suggested a mechanism for the repair of gaps opposite the pyrimidine dimers. They postulate the occurrence of "recombination-like" events after replication. In each recombination event the daughter strand containing a gap at a given locus pairs with its complementary strand, which may contain gaps elsewhere but never at the same locus since this would result in a double strand break. Such single-stranded DNA exchanges would permit repair synthesis to fill in the gap using the complete, complementary strand as a
Evidence supporting the need for recombination to correct these gaps comes from two sources. First, it has been demonstrated that the ability of an excisionless strain of *E. coli* to tolerate a single unrepaired pyrimidine dimer is completely eliminated in a recombinationless strain (Ganesan and Smith 1969; Howard-Flanders, Theriot and Stedeford 1969). Secondly, it has been shown (Howard-Flanders, Wilkins, Rupp and Cole 1968) that "post-replication" repair does not occur unless both daughter strands of DNA produced by the first post-irradiation replication are present. Howard-Flanders et al transferred irradiated F lac+ episomes from excisionless donors into Rec+ recipients. Since bacterial conjugation is a reliable way to separate the two daughter molecules the repair of the gaps opposite the pyrimidine dimers could be examined while isolated from its undamaged complementary daughter strand which would have provided a "correction" template. They found that the episomal DNA is not repaired. This leads to the conclusion that "post-replication" repair depends not only on recombination ability but also the actual presence of both daughter molecules of DNA.

**Pedigree Analysis and Recombination**

Pedigree analysis by micromanipulation is a technique that can be used to elucidate the nature of the recombination pro-
cess. This technique has not been specifically utilized to study U.V. induced mitotic recombination, but its value has been indicated by Wood (1967).

While studying the U.V. induction of recombination in germinating Aspergillus conidia, Wood found evidence that recombination may occur both at the first division following irradiation and in a subsequent division. In these cases, the production of a pigmented sector was either accompanied by an additional cross-over of a nutritional marker in its reciprocal product or both the colour sector and its reciprocal product had a nutritional requirement due to the segregation of an unlinked marker.

In terms of the proposed hybrid DNA recombination models this latter result suggests that the segregation of unrepaired hybrid DNA may occur. The use of normal plating techniques was inadequate to determine if this was the case. Wood concluded that if the segregation of unrepaired hybrid DNA was occurring, then it might be revealed by possible segregation patterns following pedigree analysis. Wood's proposed segregation patterns are diagrammed in Figures 5 and 6. Figure 5 illustrates a Type I segregation pattern such as would result from mitotic recombination involving the exchange of whole-chromatids, while Figure 6 shows one possible delayed segregation pattern (Type II) that would be expected from the segregation of unrepaired DNA following single-stranded DNA exchanges.
Figure 5. Type I Segregation Following Whole Chromatid Exchange.
Figure 6. Type II Segregation Following Half-Chromatid Exchange.
Pedigree Analysis and Lethal Sectoring

In normal plating experiments, survival is estimated by a microscopic colony count. This conceals a great deal of information concerning the fate of individual cells. When pedigree analysis is undertaken, four types of events can be distinguished. They are (1) single cells which never divide (2) cells which demonstrate only a few residual divisions and produce a microscopic clone (3) cells which produce pedigrees of macroscopic colonies only and (4) cells which produce a mixture of types 1 and 2 or 1 and 3.

In plating experiments, types 1 and 2 would be classed as non-survivors while types 3 and 4 would be indistinguishable and appear as survivors. Type 4 pedigrees contain "lethal sectors" and this has been found to represent a major form of radiation damage. In fact at X-ray giving approximately 50% survival, more than half the surviving diploid S. cerevisiae pedigrees revealed lethal sectoring (James and Werner 1966). Lethal sectoring is not restricted to yeast but has also been shown to occur in bacteria (Haefner 1967b), in algae (Marcenko 1967) and in mammalian tissue culture (Miltenburger 1966 and Thompson 1967).

The frequency of lethal sectoring decreases in each post-irradiation generation but pedigrees displaying one lethal sector are very likely to express another later in the pedigree (see James 1967 for review). Furthermore, James and Saunders (1968) have shown that these sectors are often part of an
apparently "permanent" genetic instability. There is also
evidence for a cytoplasmic effect since certain media or
strains cause preferential lethal sectoring either in the
parental or in the daughter buds (James 1967). Haefner (1965)
has demonstrated that the amount of lethal sectoring is dependent
upon the ultra-violet dose and scattered evidence suggests the
same is true for X-ray doses (James 1967). It has been shown
(Haefner 1967c) that with one exception, all ploidies of yeast
are capable of induced lethal sectoring by either ultra-violet
or X-ray exposures. The exception is that X-rays cannot induce
lethal sectoring in haploids. This is remarkable as it has
been found that dark repair does not occur after X-ray-irradiation
of haploid yeast cells (Patrick, Haynes and Uretz 1964).

There is evidence that multisite damage and repair
mechanisms are involved. James and Werner (1966) found that
the time from irradiation to the first cell division was
important in determining what type of damage would be demon-
strated. Thus, the greater the time between the irradiation and
the first division, the greater the chance of survival (survival
includes sectored plus normal colonies). James and Werner con-
cluded that there was multisite damage and that mitotic delay
increases the probability that the affected sites would be
repaired. Thus, partial repair resulted in the conversion of
lethal damage into less detrimental lethal sectors. Furthermore,
mercaptoethanol treatment, which inhibits cell division (pro-
ably allowing more time for DNA repair), was found to increase
survival from 30% to 76% and to decrease the fraction of sectored colonies among the survivors from 70% to 45% (James and Werner in press).

The persistance of X-ray induced lethal sectoring has been demonstrated by James et al (1968), who found it possible to isolate cell lines which maintained the property of producing lethal sectors as a "permanent" characteristic.

In an attempt to determine the mechanism of lethal sectoring, Haefner (1968), examined eight recombination deficient (rec-) mutants of E. coli by means of micromanipulation and pedigree analysis. The rec- strains all showed an increased level of spontaneous lethal sectoring by a factor of four to more than twenty times the rec+ "wild" type. Furthermore, a mutant gene uvrA6, which is rec+ but excisionless, doubled the spontaneous lethal sectoring frequency when present in any rec- strain.

James and Werner (1966) found a relationship between mutation and lethal sectoring. After pedigree analysis of X-ray treated diploid Saccharomyces cerevisiae, they sporulated samples of each cell line and then determined the frequency of recessive lethal mutations. About 30% of the treated cells had recessive lethal mutations but those pedigrees demonstrating lethal sectors had a significantly greater portion of the recessive lethal mutations (68% vs. 21%). However, recessive lethal mutations are not directly responsible for lethal sectors
(James 1967). The segregation of induced recessive lethal mutations occur almost entirely in the first division following irradiation (Haefner 1967; James and Werner 1966). This behaviour is unlike lethal sectoring which, as stated earlier, may persist over many generations.

From the above review, it appears that repair and recombination are involved in lethal sectoring. In the present study, these relationships were investigated using strains deficient in either recombination or radiation repair capability. As well, a number of U.V. sensitive mutants of *Saccharomyces cerevisiae* exist (Snow 1968). X-ray sensitive mutations are also available (Nakai and Matsumoto 1967). An X-ray resistant strain of *S. cerevisiae* has been isolated by Moustacchi (1965). These strains present a good opportunity to examine the relationship between radiation repair, lethal sectoring and recombination.

However, the main purpose of the present study was to develop a system in which the descent of the nuclear products could be followed and recognized. *Saccharomyces cerevisiae* seemed useful for this purpose since it offers the following advantages: (1) it is quite well mapped (for a recent map see Hawthorne and Mortimer 1968), (2) it is a single celled organism which produces stable diploids which can go through meiosis producing a tetrad, (3) it can be studied successfully by pedigree analysis (Tobias, Mortimer, Gunther and Welch 1958; Haefner 1965; James and Werner 1966 a, b; James
1967; and James and Saunders 1968).
MATERIALS AND METHODS

Strains

Strains of *Saccharomyces cerevisiae* were used in all experiments. The genetic markers are described by conventions adopted at the Carbondale Yeast Genetics Conference (von Borstel 1961). Upper and lower case letters for a gene symbol indicate the dominant and recessive alleles respectively.

**D3:** This strain was the diploid used in all the recombination experiments and was supplied by F.K. Zimmerman, at the University of Freiberg, in Freiberg, Germany. Strain D3 was heterothallic and heterozygous at eight loci on four chromosome arms. The mutants and corresponding requirements (when homozygous for the recessive alleles) were: ad₂, adenine; hi₈, histidine; ur₃, uracil; thr₃, threonine; is₁, isoleucine and valine; tr₂, tryptophane; val, valine and isoleucine. The mutant Acᵣ was dominant and conferred resistance to actidione. The meiotic map for strain D3 is given in Figure 7.

**842-3A:** This strain was an X-ray sensitive recombination deficient haploid supplied by Dr. S. Nakai at the National Institute of Radiological Sciences in Chiba-Shi, Japan. Strain 842-3A had the following markers: 'a' mating type; X₁ˢ, X-ray sensitive; ar₄, arginine; hi₅, histidine; ly₁, lysine; ad₂, adenine; le₁, leucine; tr₅, tryptophane. The mutation X₁ˢ is classed as recombination deficient because when homozygous
Figure 7. Marker arrangement in Strain D3. The number in parenthesis represent the distance between markers in meiotic map units. These distances were derived from a map produced by Mortimer and Hawthorne (1966). The open circles indicate the location of the centromeres.
in a diploid strain, it prohibits X-ray induced mitotic recombination without affecting spontaneous mitotic recombination.

277-lb: Strain 227-lb was a haploid obtained from R. Snow at the University of California, Davis, California. The strain genotype was: 'α' mating type; uvs-9. The uvs-9 mutant was originally isolated by Snow (1967) and results in U.V. sensitivity.

N123-P4 and 10018-P10: Both these strains are X-ray resistant and were isolated by E. Moustacchi (1965) at the Pasteur Institute, Paris, France. Their genotypes are: N123-P4-'a' mating type; hi₁, histidine; Xᵣ, X-ray resistant; 10018-P10-'α' mating type; ad₁, adenine; Xᵣ, X-ray resistant. The mutations were produced by the decay of $^{32}$P which had been incorporated into the DNA of the parent strains. They are either allelic or identical mutations.

M21-3D: This strain is the "wild type" strain used as a control and was mating type 'a'. Strain M21-3D was supplied by A.P. James at Chalk River, Ontario, but was originally from the yeast collection of Berkeley, California.

Media

Complete Media: A fortified media was used which contained:

(NH₄)₂SO₄, 0.6%; MgSO₄, 0.1%; KH₂PO₄, 0.2%; Bacto-peptone, 0.5%; Difco Yeast Extract, 0.25%; Dextrose, 2.0%; and agar, 2.0%.
(For liquid complete medium, the agar was omitted.) The dextrose was added just prior to use.

**Minimal Medium:** The minimal medium was composed of: Difco Yeast Nitrogen Base without amino acids, 0.67%; dextrose, 4.0%; and asparagine, 0.2%.

**Test Media:** The following were added to the minimal medium to test cell lines for nutrient requirements: 20 mg./liter valine; 100 mg./liter filter sterilized threonine. A cell was tested for a specific nutrient by plating it on test medium which omitted the specific nutrient.

**Actidione Resistance Test Medium:** The complete medium was supplemented with 5 mg./liter of actidione.

**Pre-Sporulation Medium:** This medium was composed of: "V-8" juice, 560 ml.; yeast extract, 0.9%; dextrose, 1.8%; and agar, 1.6%. The pH was adjusted to about 7.0 with 5N KOH.

**Sporulation Medium:** The composition of the sporulation medium was as follows: potassium acetate, 1.0%; yeast extract, 0.25%; dextrose, 0.1%; and agar, 1.5%.

**Procedures**

**Pre-treatment Conditions:** Early stationary phase cells were used in all experiments. The following procedure was used to produce these cells: Ten ml. of liquid complete medium was
inoculated and grown over-night at 30°C in a shaker waterbath. This culture was then standardized in fresh liquid complete medium to produce 50% absorbance at 400μm on a Bausch and Lamb Spectronic 20. From this standardized culture, 0.1 ml. was used to inoculate 30 ml. of liquid complete medium. This culture was then incubated at 30°C in a shaker water-bath for 48 hours. The cells were then harvested and washed twice in sterile, double distilled and deionized water. The cells were then resuspended in 60 ml. of distilled water. This produced a suspension of about 10^8 cells/ml. which was the concentration used in all ultra-violet experiments. A concentration of 10^7 cells/ml. was used for the X-ray experiments.

**Ultra-violet Light Treatment:** A General Electric 15 watt germicidal lamp producing 95% of its light energy at 2537 Å was used as an ultra-violet light source. The U.V. irradiation was monitored by an International Light Inc. UV254 U.V. meter, and the dose was controlled by a digital integrator which was developed by R. Osborne (1969). The dose rate was 14 ergs/mm.²/sec. except when the total dose was less than 200 ergs/mm.². In the latter case, a fine copper screen reduced the dose rate to 4 ergs/mm.²/sec.

Five ml. samples were irradiated in 15 mm. deep petri dishes. The samples were constantly agitated during the irradiation by means of an asynchronous motor which was mounted on the
stand holding the petri dish. Precautions were taken against photoreactivation by using yellow room-lighting and storing the irradiated samples in red test tubes.

**Photoreactivation:** Photoreactivation was accomplished by using a high intensity air cooled mercury lamp (General Electric B-HG). Mercury lamps have a broad spectrum output including U.V. and infra-red wavelengths. The U.V. and infra-red light produced by this lamp was removed by the 5% CuSO₄ solution present in the cooling bath. The sample tubes were kept at 25°C for the duration of the high intensity light treatment.

**X-ray Treatments:** A 2 MeV X-ray generator was used for the X-ray treatments. The dose rate, 5.9 cm. from the target was 10,000 R/minute. Nine ml. samples were irradiated in aluminum capsules spinning at 100 r.p.m. An internal fixed rotor kept the samples well mixed.

**Pedigree Analysis**

Cells for pedigree analysis were isolated on a sterile slab of complete medium. The isolation and separation of individual cells was done with a de Fonbrune micromanipulator using a glass needle manufactured upon a de Fonbrune micro-forge.

The agar slabs were prepared in a manner described by Haefner (1967a) using a duralum or brass box that produced
slabs of even thickness (approximately 2 mm.). These slabs were placed upon sterile slides which were then set on plastic chambers and sealed with vaseline. The chambers contained enough distilled water to keep the agar from drying out.

The isolated cells were placed on the agar slab at premarked locations. The slides were then incubated at 30°C and checked regularly to follow growth.

When divisions occurred, daughter cells were separated from their mother and moved to predetermined locations.

In this manner 100-300 cells could be handled in a lethal sectoring experiment that was carried to only one division. During recombination experiments the pedigrees were taken to the eight cell stage whenever possible. Fifty treated cells was the maximum number which could be managed in one experiment. Pedigree analysis during the recombination experiments was continued for 24 hours but at the end of this period many pedigrees were not at the eight cell stage. The incomplete pedigrees were due to (1) mitotic delay (2) lack of replication ability and (3) loss of cells due to technical difficulties such as misplacing cells in the agar or not separating mother and daughter cells before a further division, therefore losing track of the lines of descent.

Identification of Recombinants:-During the study of mitotic recombination by pedigree analysis, recombinant pedigrees were detected as follows: after the pedigree products had been
incubated for 2 to 3 days, the colonies were transferred by means of a flamed platinum loop (gauge 23) onto both minimal and complete media plates. This operation was carried out at a Liminal Flow clean station (from Controlled Environmental Equipment Corporation, Whitham, Mass., U.S.A.).

When the transferred colony failed to grow on minimal medium, the corresponding colony on the complete medium plate and all other cell lines in that pedigree were replicated upon the omission media. The phenotype of the colonies was determined by this procedure.

**Genetic Analyses**

**Tetrad Analysis**:- The sporulation of diploid cell lines was promoted by first growing the cells over-night at $30^\circ$C on V-8 medium and then transferring them to the potassium acetate slants. These slants were allowed to dry at an angle that left the innoculum well spread upon the slant's surface. The tubes were kept at room temperature ($25^\circ$C) and after three days were examined for the presence of asci.

When the sample had sporulated, a heavy loopful was transferred to 1 ml. of Helicase solution (supplied by Industrie Biologique Française, Siene, France) which digested the ascus wall. After the digestion had proceeded far enough to allow easy dissection of the ascospores (25-50 minutes), the spore tetrads were isolated onto complete media agar slabs.
The spores were then separated and placed in pre-determined positions and incubated at 30°C until colonies 2-3 mm. in diameter were present. Cells from these were transferred to slabs of omission media where growth was observed with a microscope.

**Induction Analysis:** When tetrad analysis was not successful (usually because of poor sporulation or non-viable spores), a second method was used to obtain information about a cell's genotype. This method, which has been termed induction analysis, could determine if a cell was homozygous or heterozygous at given loci.

The procedure was to treat the cell line in question with a U.V. dose of 1500 ergs/mm.² followed by 7.5 minutes visible light. The cells were then plated on complete medium at a dilution which produced 100-200 colonies per plate. If among 1000-2000 colonies, none required a specific nutrient, the cell line was said to be homozygous for the "wild type" allele at that locus. On the other hand, if colonies demonstrated a requirement for that specific nutrient, the cell line was assumed to be heterozygous at that locus. This method is highly discriminating as controls using cell lines known to be homozygous and heterozygous for specific markers, showed that the difference was absolute, i.e. if the cell line was homozygous no requiring colonies were found, while if the cell line was heterozygous, at least 25 requiring colonies were identified.
Since the is$_1$ marker produced a requirement for both isoleucine and valine, as did the valine marker, these were distinguished on the basis of accompanying requirements caused by linked markers.
RESULTS

I. A Study of Mitotic Recombination by Pedigree Analysis

Preliminary Experiments

The initial experiments were designed to determine the best ultra-violet and post-irradiation treatment which would induce mitotic recombination and also facilitate pedigree analysis. For efficient pedigree analysis a survival of about fifty percent of the cells was considered desirable.

Survival After U.V.: - The survival of the diploid strain D3 following various doses of U.V. is given in Figure 8. Doses greater than 2500 ergs/mm.$^2$ were not tested since cell survival would be too low to permit pedigree analysis.

Photoreactivation: - The effect of visible light treatments of varying lengths, following a U.V. dose of 750 ergs/mm.$^2$ is illustrated in Figure 9. The optimum duration of the visible light treatment was found to be 7.5 minutes. This exposure resulted in a survival of 61%, about twice that of the untreated samples.

It was observed that longer periods of visible light treatment decreased survival. In fact, 15 minutes exposure to visible light reduced survival to 36%.
Figure 8. The survival curves for the diploid Saccharomyces cerevisiae strain D3 with and without 7.5 minutes visible light treatment is illustrated above. The standard error of the mean is given.
Figure 9. The effect of visible light upon survival. The cells were pretreated with 750 ergs/mm$^2$ U.V. The optimum length of the visible light treatment was determined to be 7.5 minutes. The standard errors of the mean are shown.
Survival After Visible Light Exposure of U.V. Treated Cells:—
The survival curve for U.V. irradiated cells when exposed to 7.5 minutes of visible light is illustrated in Figure 8. A U.V. irradiation of 1500 ergs/mm.\(^2\) followed by 7.5 minutes exposure to visible light was the highest dose allowing sufficient survival for efficient pedigree analysis. Further investigation of the effect of this proposed treatment was deemed necessary.

The U.V. Induction of Mitotic Crossing-Over:— It is known that U.V. induces recombination and in addition it has been reported that photoreactivation increases cell survival without affecting the amount of recombination. Before using pedigree analysis to study recombination, it was desirable to obtain some data for the proposed treatment from standard plating experiments. In this way, the amount of recombination, if any, which would result from this treatment would be determined. Secondly, the plating experiments would provide a basis for comparison with the results obtained from pedigree experiments.

The amount of mitotic recombination induced by a U.V. irradiation of 1500 ergs/mm.\(^2\) was determined by the segregation of a visible marker (ad\(_2\)) present in the strain D3. Pink colonies are produced by ad\(_2\) homozygous cells due to a metabolic block which results in the accumulation of a red pigment. Data for
both photoreactivated and non-photoreactivated cells can be found in Table II.

<table>
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<th>1500 ergs/mm$^2$</th>
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<tr>
<td>Photoreactivation</td>
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<td>Survivals</td>
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<td>15</td>
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<td>No. Recombinants</td>
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<tr>
<td>&quot;mosaic&quot;-red &amp; white</td>
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<td>129</td>
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<td></td>
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<td>144</td>
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<td>% Recombinants</td>
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<tr>
<td>among survivors</td>
<td>4.7%</td>
<td>4.8%</td>
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Table II. Induced Mitotic Crossing-Over in Strains D3: Data from Plating Experiments.

Photoreactivation appeared to have no effect upon the total frequency of crossing-over. The amount of recombination found in both the photoreactivated and non-photoreactivated cells was identical. However, photoreactivation had a pronounced effect upon the type of recombinant colonies observed.

The recombinant colonies were classed into two groups, those which were red-white mosaics and those which were pure red. The photoreactivated sample had a far greater proportion of mosaic
colonies (9:1) than the non-photoreactivated sample where the ratio was 1:1. This difference is highly significant ($p < 10^{-12}$).

Since the visible light treatment produced more of the mosaic colonies expected from intergenic crossing-over, as well as increased survival, this treatment was most valuable to the pedigree analysis. The U.V. dose of 1500 ergs/mm$^2$ followed by 7.5 minutes of visible light treatment was used as the standard procedure for studying induced mitotic recombination by pedigree analysis.

**Pedigree Analysis of Strain D3:** The behaviour of strain D3 during pedigree analysis was first examined in non-irradiated cells. Fifty pedigrees were carried to the third generation, or the eight cell stage. This would have produced a total of 400 colonies but as there were two lethal sectors, both in the third generation, there were only 398 colonies. Thus the spontaneous lethal sectoring frequency was 0.05%. All the original isolates produced colonies and therefore no deaths were attributable to the isolation procedure. All of the 398 colonies grew when transferred to plates of minimal medium and thus none of the pedigree products were recombinants.

**The Study of U.V. Induced Mitotic Recombination by Pedigree Analysis:** Pedigree analysis of 150 U.V. irradiated and photoreactivated cells were carried out. Of these 46 died. Among the remaining pedigrees, 18 displayed recombination. These 18 pedigrees are shown in Figures 10 to 28 along with their
phenotypes and genotypes.

Recombinants could be identified by their nutritional requirements. Strain D3 carried seven nutritional markers which are present in four of the linkage groups. Recombinants which are homozygous for any of these markers will no longer grow on media without the appropriate nutritional supplements. None of the colonies required any other nutrients than those which could be predicted from a knowledge of the seven nutritional markers present in strain D3. Thus, no new mutants were detected.

The genotypes of the recombinant cells were determined. Tetrad analysis is the most precise way of determining a genotype. Unfortunately, several attempts were often required to obtain even a single viable spore. When all attempts at tetrad analysis failed, induction analysis was used.

Segregation in Recombinant Pedigrees:—Upon examining the 18 recombinant pedigrees, it can be seen that a total of 22 chromosome arms showed recombination. Segregation was restricted to the first post-irradiation generation in 18 of the 22, and these were classed as Type I segregants (for a description of Type I and Type II segregation patterns see pp. 22). The other four pedigrees are reported below.

Pedigree 15:—In this pedigree, the linked markers is₁ and tr₂ segregated in the second generation on both sides of the pedigree (Figure 12). This pedigree could be classified as
Introduction to the Recombinant Pedigrees

Pedigree Products:

Symbols:
- X = a lethal sector.
- + = Cell-line will grow in the absence of a specific nutrient.
- - = Cell-line requires a specific nutrient.
- R = Cell-line resistant to 5 mg/l of actidione.
- S = Cell-line sensitive to 5 mg/l of actidione.

Genotypes:
- T = genotype determined by tetrad analysis.
- I = genotype determined by induction analysis.
- A = genotype assumed.
- () = Cell-line lost in lethal sector, genotype inferred.

Figure 10.
Recombinant Pedigree 5

Phenotype:

\[
\begin{array}{cccccccc}
\text{Ac}^R & R & R & R & R & R & R & R \\
\text{ad} & + & + & + & - & - & - & - \\
\text{hi} & + & + & + & - & - & - & - \\
\end{array}
\]

Genotype:

\[
\begin{array}{cccccccc}
\frac{\text{Ac}^R}{\text{ac}^R} & \frac{\text{Ac}^R}{\text{ac}^R} & \frac{\text{Ac}^R}{\text{ac}^R} & \frac{\text{Ac}^R}{\text{ac}^R} & \frac{\text{Ac}^R}{\text{ac}^R} & \frac{\text{Ac}^R}{\text{ac}^R} \\
\text{Ad} & \text{Ad} & \text{Ad} & \text{ad} & \text{ad} & \text{ad} \\
\text{Ad} & \text{Ad} & \text{Ad} & \text{ad} & \text{ad} & \text{ad} \\
\text{Hi} & \text{Hi} & \text{Hi} & \text{hi} & \text{hi} & \text{hi} \\
\text{Hi} & \text{Hi} & \text{Hi} & \text{hi} & \text{hi} & \text{hi} \\
\end{array}
\]

Method:

\[
\begin{array}{cccccccc}
I & I & A & A & A & I \\
\end{array}
\]

Figure 11.
Recombinant Pedigree 15

Phenotype
thr  +  +  +  +
is-val +  -  +
tr   +  -  +

Genotype:

\[
\begin{array}{cccc}
\text{Thr} & \text{Thr} & \text{thr} & \text{thr} \\
\text{thr} & \text{thr} & \text{Thr} & \text{Thr} \\
\text{is} & \text{Is} & \text{is} & \text{Is} \\
\text{is} & \text{Is} & \text{is} & \text{Is} \\
\text{tr} & \text{Tr} & \text{tr} & \text{Tr} \\
\text{tr} & \text{Tr} & \text{tr} & \text{Tr} \\
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Method: A  I  I  I

Figure 12.
Recombinant Pedigree 40

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Figure 13.
Recombinant Pedigree 41

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Method: A A A A A T T A T

Figure 14.
Recombinant Pedigree 45

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Genotype:

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Thr  Thr  Thr  thr  thr
Is   Is   Is   is   is
Is   Is   Is   is   is
Tr   Tr   Tr   tr   tr
Tr   Tr   Tr   tr   tr
```

Method: T I I A A

Figure 15.
Recombinant Pedigree

Phenotype:

\[ Ac^r \quad S \quad R \quad R \]

\[ Ad \quad + \quad - \quad + \]

\[ hi \quad + \quad - \quad + \]

Genotype:

\[
\begin{array}{ccc}
\frac{acr}{acr} & \frac{Ac^r}{acr} & \frac{Ac^r}{acr} \\
\frac{Ad}{ad} & \frac{ad}{ad} & \frac{Ad}{Ad} \\
\frac{Hi}{hi} & \frac{hi}{hi} & \frac{Hi}{hi} \\
\end{array}
\]

\[
\begin{array}{ccc}
\frac{(Ac^r)}{Ac^r} & \frac{(Ad)}{ad} & \frac{(Hi)}{Hi} \\
\end{array}
\]

Method:

I \ \ I \ \ I

Figure 16.
Recombinant Pedigree 71

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Method: T A I A I I
Recombinant Pedigree 73

Phenotype:

\( \text{Ac}^r \)  \( \text{R} \)  \( \text{S} \)  \( \text{R} \)
\( \text{ad} \)  -  -  +
\( \text{hi} \)  +  +  +

Genotypes:

\[
\frac{\text{Ac}^r}{\text{acr}} \quad \frac{\text{acr}}{\text{acr}} \quad \frac{\text{Ac}^r}{\frac{\text{acr}}{\text{acr}}} \\
\frac{\text{ad}}{\text{ad}} \quad \frac{\text{ad}}{\text{ad}} \quad \frac{\text{Ad}}{\frac{\text{Ad}}{\text{Ad}}} \\
\frac{\text{hi}}{\text{Hi}} \quad \frac{\text{hi}}{\text{Hi}} \quad \frac{\text{Hi}}{\frac{\text{Hi}}{\text{hi}}} \\
\]

Method:

I  I  I  A

Figure 18.
Recombinant Pedigree 74

Phenotype:

\[ \text{Ac}^r \]
\[ \text{Ad} \]
\[ \text{hi} \]

Genotypes

\[ \text{(?)} \]
\[ \text{?} \]

\[ \frac{\text{Ad}}{\text{Ad}} \]
\[ \text{ad} \]
\[ \text{ad} \]

\[ \frac{\text{Hi}}{\text{Hi}} \]
\[ \text{hi} \]
\[ \text{hi} \]

Method:

A

Figure 19.
Recombinant Pedigree 98

Phenotype:

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Genotype:

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\text{Is} & \text{Is} & \text{Is} & \text{Is} & \text{Is} & \text{Is} & \text{is} \\
\text{Is} & \text{Is} & \text{Is} & \text{Is} & \text{Is} & \text{is} & \text{is} \\
\text{Tr} & \text{Tr} & \text{Tr} & \text{Tr} & \text{Tr} & \text{tr} & \text{tr} \\
\text{Tr} & \text{Tr} & \text{Tr} & \text{Tr} & \text{Tr} & \text{tr} & \text{tr} \\
\end{array}
\]

Method: T A T A A A

Figure 20.
Recombinant Pedigree 107

Phenotype:

- Val
- Is
- Ac^r
- ad
- hi

Genotype:

\[
\text{val} \quad \text{val} \quad \text{val} \quad \text{val} \quad \text{Val} \\
\text{val} \quad \text{val} \quad \text{val} \quad \text{val} \quad \text{Val} \\
\text{Ac}^r \quad \text{Ac}^r \quad \text{Ac}^r \quad \text{Ac}^r \quad \text{Ac}^r \\
\text{acr} \quad \text{acr} \quad \text{acr} \quad \text{acr} \quad \text{acr} \\
\text{ad} \quad \text{ad} \quad \text{ad} \quad \text{ad} \quad \text{ad} \\
\text{Ad} \quad \text{Ad} \quad \text{Ad} \quad \text{Ad} \quad \text{Ad} \\
\text{hi} \quad \text{hi} \quad \text{hi} \quad \text{hi} \quad \text{hi} \\
\text{Hi} \quad \text{Hi} \quad \text{Hi} \quad \text{Hi} \quad \text{Hi} \\
\text{Method: I} \quad \text{T} \quad \text{A} \quad \text{T} \quad \text{A} \\
\]

Figure 21.
Recombinant Pedigree 111

Phenotype:
\[ \begin{array}{cccccc}
Ac^r & S & S & S & R & R & R & R & R \\
ad & + & + & + & - & - & - & - & - \\
hi & + & + & + & - & - & - & - & - \\
\end{array} \]

Genotype:
\[ \begin{array}{cccccccc}
\frac{ac^r}{ac^r} & \frac{ac^r}{ac^r} & \frac{ac^r}{ac^r} & \frac{Ac^r}{Ac^r} & \frac{Ac^r}{Ac^r} & \frac{Ac^r}{Ac^r} & \frac{Ac^r}{Ac^r} \\
Ad & Ad & Ad & ad & ad & ad & ad & ad \\
\frac{Ad}{Ad} & \frac{Ad}{Ad} & \frac{Ad}{Ad} & \frac{ad}{ad} & \frac{ad}{ad} & \frac{ad}{ad} & \frac{ad}{ad} & \frac{ad}{ad} \\
Hi & Hi & Hi & hi & hi & hi & hi & hi \\
\frac{Hi}{Hi} & \frac{Hi}{Hi} & \frac{Hi}{Hi} & \frac{hi}{hi} & \frac{hi}{hi} & \frac{hi}{hi} & \frac{hi}{hi} & \frac{hi}{hi} \\
\end{array} \]

Method: A I A A A I A

Figure 22.
Phenotype:

Ac<sup>r</sup>  R   R   R   R   S   S   S   S   S
ad     -   -   -   -   +   +   +   +   +
hi     -   -   -   -   +   +   +   +   +

Genotype:

\[
\begin{array}{cccccccc}
\text{Ac}<sup>r</sup> & \text{Ac}<sup>r</sup> & \text{Ac}<sup>r</sup> & \text{Ac}<sup>r</sup> & \text{ac}<sup>r</sup> & \text{ac}<sup>r</sup> & \text{ac}<sup>r</sup> & \text{ac}<sup>r</sup> \\
\hline
\text{ad} & \text{ad} & \text{ad} & \text{ad} & \text{Ad} & \text{Ad} & \text{Ad} & \text{Ad} \\
\text{ad} & \text{ad} & \text{ad} & \text{ad} & \text{Ad} & \text{Ad} & \text{Ad} & \text{Ad} \\
\text{hi} & \text{hi} & \text{hi} & \text{hi} & \text{Hi} & \text{Hi} & \text{Hi} & \text{Hi} \\
\text{hi} & \text{hi} & \text{hi} & \text{hi} & \text{Hi} & \text{Hi} & \text{Hi} & \text{Hi} \\
\end{array}
\]

Method: A   A   A   A   A   A   T   A   A

Figure 23.
Phenotype: val

Genotype: val

Method: A

Figure 24.
Recombinant Pedigree 121

Phenotype:

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Genotype:

\[
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\text{thr} & \text{thr} & \text{thr} & \text{thr} & \text{thr} & \text{Thr} & \text{thr} \\
\text{is} & \text{is} & \text{is} & \text{is} & \text{Is} & \text{Is} & \text{Is} \\
\text{is} & \text{is} & \text{is} & \text{is} & \text{Is} & \text{Is} & \text{Is} \\
\text{tr} & \text{tr} & \text{tr} & \text{tr} & \text{Tr} & \text{Tr} & \text{Tr} \\
\text{tr} & \text{tr} & \text{tr} & \text{tr} & \text{Tr} & \text{Tr} & \text{Tr} \\
\end{array}
\]

Method: T T A A I I

Figure 25.
Recombinant Pedigree 133

Phenotype:

\[ \text{Ac}^r \quad \text{R} \\
\text{ad} \quad - \\
\text{hi} \quad - \\

\text{Genotype:} \\

\begin{align*}
\text{Ac}^r & \quad ? \\
\text{ad} & \quad \text{ad} \\
\text{hi} & \quad \text{hi} \\
\end{align*}

Method: A

Figure 26.
Recombinant Pedigree 138

Phenotype:

\[
\begin{array}{cccccc}
\text{Ac}^r & \text{R} & \text{R} & \text{R} & \text{R} & \text{R} \\
ad & + & + & + & + & + \\
hi & + & - & - & - & - \\
ur & + & - & - & - & - \\
\text{thr} & + & + & + & + & + \\
\text{ls-val} & - & + & + & + & + \\
\text{tr} & - & + & + & + & + \\
\end{array}
\]

Genotype:

\[
\begin{array}{cccccccc}
\frac{\text{Ac}^r}{\text{ac}^r} & \frac{\text{Ac}^r}{\text{Ac}^r} & \frac{\text{ac}^r}{\text{Ac}^r} & \frac{\text{ac}^r}{\text{Ac}^r} & \frac{\text{ac}^r}{\text{Ac}^r} \\
\text{Ad} & \text{ad} & \text{ad} & \text{ad} & \text{ad} \\
\text{ad} & \text{Ad} & \text{Ad} & \text{Ad} & \text{Ad} \\
\frac{\text{Hi}}{\text{hi}} & \frac{\text{hi}}{\text{hi}} & \frac{\text{hi}}{\text{hi}} & \frac{\text{hi}}{\text{hi}} & \frac{\text{hi}}{\text{hi}} \\
\frac{\text{Ur}}{\text{ur}} & \frac{\text{ur}}{\text{ur}} & \frac{\text{ur}}{\text{ur}} & \frac{\text{ur}}{\text{ur}} & \frac{\text{ur}}{\text{ur}} \\
\text{Thr} & \text{thr} & \text{thr} & \text{thr} & \text{thr} \\
\text{thr} & \text{Thr} & \text{Thr} & \text{Thr} & \text{Thr} \\
\text{is} & \text{Is} & \text{Is} & \text{Is} & \text{Is} \\
\text{is} & \text{Is} & \text{Is} & \text{Is} & \text{Is} \\
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Method:

I I A I A

Figure 27.
Recombinant Pedigree 146

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</table>

Method: T A A A A A A T A

Figure 28.
having two Type I segregations in the second generation or a modification of the Type II segregation pattern. Non-disjunction can be ruled out by the behaviour of the linked marker THR and the marker ur, on the other side of the centromere.

Pedigree 54:-In this pedigree, although there was a second generation lethal sector, it was observed that the linked markers, AC⁴, ad₂ and hi₈ of Fragment 1, segregated in the second post-irradiation generation in a Type II pattern (Figure 16). The fact that the three linked markers segregated in a Type II fashion is not predicted by the "uncorrected hybrid DNA" segregations postulated by Wood (1967).

Pedigree 71:-The valine marker segregated in a non-reciprocal manner in the second generation of the pedigrees (Figure 17). Since the is₁ gene had segregated in the first generation after irradiation, the fate of the valine marker on that side of the pedigree is unknown. However, as the valine recombination event was non-reciprocal its segregation seems to best fit a Type II pattern. There was a second generation lethal sector in this pedigree.

Pedigree 73:-This pedigree (Figure 18) was very similar to pedigree 54 as there was (1) a lethal sector in the second generation and (2) the linked markers of Fragment 1 segregated in a Type II fashion. This pedigree differed from pedigree 54 as only the AC⁴ and ad₂ alleles segregated. The hi₈ allele remained heterozygous.
Frequencies of Induced Mitotic Recombination:—Table III gives the frequencies of induced mitotic recombination at each locus and in each linkage group. Comparing the results for the $a d_2$ locus from pedigrees and from plates, it is observed that the recombination frequencies determined by pedigree analysis were higher (9% vs. 4.8%). Presumably pedigree analysis allows the examination of pedigree products with a much greater resolution. Pedigree 107, Figure 21, is a good example. In this pedigree the homozygous recessive genotypes were most probably in a lethal sector.

Coincidence of Cross-Overs in Different Linkage Groups:—A comparison between the expected and observed coincidence of U.V. induced mitotic cross-overs in different linkage groups is given in Table IV. Although the numbers are too low to permit statistical analysis, the first analysis (A) suggested that coincidence was high. Further examination, however, revealed that six of the seven multiple events occurred in two of the pedigrees which each show crossing-over in three linkage groups. When these pedigrees are removed from the data, any evidence of coincidence disappears (Table IV part B). This analysis suggests that the population may be heterogenous, and that one population produces pedigrees containing multiple events (C).

Mitotic Delay:—The U.V. treatment caused a delay in the first division times of the cells. In the control sample the first
## Table III. Effect of U.V. upon Mitotic Recombination: Data from Pedigrees.

<table>
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<td>5</td>
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<td>146</td>
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</tbody>
</table>

### Recombinant Total for each locus:
- 4 8 9 3 9 9 2 3

### % Recombination Among Survivors
- 4% 8% 9% 3% 9% 9% 2% 3%

### Total Recombinants for each arm:
- 9 9 2 3

### % Recombination Among Survivors
- 9% 9% 2% 3%

### Recombinant Pedigree Identification Number
- 54 54 54 40 40 138 107
- 73 73 73 41 41 116
- 74 74 45 45 45
- 107 107 71 71
- 111 111 98 98
- 112 112 121 121
- 133 133 138 138
- 138 146 146 146
## COINCIDENCE OF U.V. INDUCED MITOTIC CROSSING-OVER

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<th>I &amp; III</th>
<th>I &amp; IV</th>
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Table IV. (A) Unadjusted data. (B) The data adjusted by removing the two pedigrees containing the triple events. (C) Coincidence of crossing-over in three linkage groups.
division usually occurred after about two hours. The mean time to division in the irradiated sample was 11.7 hours.

When the comparison was made between the first division times of the normal pedigrees and those containing recombinant products, no difference was detected (Figure 29 A). Both classes of pedigrees had a mitotic delay of about 11 hours. Pedigrees displaying lethal sectors were found to have a mean division time of 13.5 hours but the difference between this and the normal mitotic delay was not significant (Figure 29 B).

**Frequency of Induced Lethal Sectoring:** The frequencies of induced lethal sectoring were much higher in all three post-irradiation generations than the spontaneous lethal sectoring frequency in the controls. This data is summarized in Table V.

Mother and daughter cells were identified at the first separation. Eighteen of the 21 first generation lethal sectors occurred in cells identified as daughters. This was significantly different (p<0.05) from random occurrence. Clearly lethal sectoring in the first generation thus tends to occur in daughter cells.

**Lethal Sectoring and Mitotic Recombination:** Since the causes of lethal sectoring are obscure, it was considered worth while to see of a relationship existed between lethal sectoring and recombination.

The relationship between recombination and lethal sectoring in
A. Recombinant vs. Non-recombinant Pedigrees.

B. Lethal Sectored vs. Non-lethal Sectored Pedigrees.

Figure 29. The distribution of first post-irradiation division times. A. Comparison of normal vs. recombinant pedigrees. B. Non-lethal sectored vs. lethal sectored pedigrees.
A. Treatment

<table>
<thead>
<tr>
<th>Survival</th>
<th>none</th>
<th>1500 ergs/mm.² and photoreactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>50/50</td>
<td>104/150</td>
</tr>
<tr>
<td>Lethal Sectoring in 1st Generation</td>
<td>0/100</td>
<td>21/202</td>
</tr>
<tr>
<td>%</td>
<td>0.0</td>
<td>10.4</td>
</tr>
<tr>
<td>Lethal Sectoring in 2nd Generation</td>
<td>0/200</td>
<td>8/122</td>
</tr>
<tr>
<td>%</td>
<td>0.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Lethal Sectoring in 3rd Generation</td>
<td>2/400</td>
<td>4/80</td>
</tr>
<tr>
<td>%</td>
<td>0.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

B. | Observed | Expected | Totals |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>3</td>
<td>10.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Daughters</td>
<td>18</td>
<td>10.5</td>
<td>28.5</td>
</tr>
<tr>
<td>TOTALS</td>
<td>21</td>
<td>21.0</td>
<td>42.0</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 4.612 \]

\[ p<0.05 \]

Table V. Lethal sectoring in strain D3. A. U.V. induced vs. spontaneous lethal sectoring. B. Distribution of first generation lethal sectors; mothers vs. daughters.
First Generation Lethal Sectoring:

<table>
<thead>
<tr>
<th></th>
<th>No. Rec.</th>
<th>Rec.</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.S.</td>
<td>18</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>No L.S.</td>
<td>65</td>
<td>15</td>
<td>80</td>
</tr>
<tr>
<td>Totals</td>
<td>83</td>
<td>18</td>
<td>101</td>
</tr>
</tbody>
</table>

\[ x^2_Y = 0.226 \]

\[ p \approx 0.64 \]

Second Generation Lethal Sectoring:

<table>
<thead>
<tr>
<th></th>
<th>No. Rec.</th>
<th>Rec.</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.S.</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>No L.S.</td>
<td>42</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>Totals</td>
<td>46</td>
<td>15</td>
<td>61</td>
</tr>
</tbody>
</table>

\[ x^2 = 3.2 \]

\[ p \approx 0.07 \]

Third Generation Lethal Sectoring:

<table>
<thead>
<tr>
<th></th>
<th>No. Rec.</th>
<th>Rec.</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.S.</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>No L.S.</td>
<td>35</td>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>Totals</td>
<td>35</td>
<td>5</td>
<td>40</td>
</tr>
</tbody>
</table>

\[ x^2_Y = 22.85 \]

\[ p < 10^{-6} \]

Table VI. Lethal Sectoring and recombination. Symbols: \( x^2 \) = Chi square, \( x^2_Y \) = Chi square with Yates' correction for continuity in small samples. L.S. = lethal sector and Rec. = a recombinant containing pedigree.
the first, second and third generations was examined. The data is presented in Table VI. First generation lethal sectors were found to be independent of recombination ($p \approx 0.64$). This comparison, however, contains a bias as some recombinants would not be detected because of the loss of the prototrophic portion in the primary lethal sector.

Recombination and lethal sectoring in the second generation appeared to be related. The pedigrees demonstrating recombination contained second generation lethal sectors three times as often as the general population (27% vs. 9%). Lethal sectoring in the third generation was closely correlated with recombination. All of the four third generation lethal sectors that were detected, occurred in pedigrees displaying recombination. The probability that these were independent events was $<10^{-6}$ when calculated by Chi square with Yate's correction for continuity.
II. Lethal Sectoring in Haploid Strains With Altered Radiation Sensitivity

Survival Curves:—The haploid strains were examined for their radiation sensitivity. The strains examined and their expected properties are listed: M21-3D, "wild type"; 227-lb, U.V. sensitive; 842-3A, X-ray sensitive, recombination deficient; N123-P4 and 10018-P10, X-ray resistant.

The survival of the "wild type" strain, M21-3D and the U.V. sensitive strain, 227-lb after U.V. treatment is given in Figure 30. The dose modification factor (DMF) for the U.V. sensitive strain (or the factor by which the dose must be decreased to produce the same survival as the "wild type" strain) is about ten. For example, the survival of the "wild type" strain, M21-3D, after a dose of 1000 ergs/mm.² was the same as the survival of strain 227-lb after a dose of only 100 ergs/mm.².

Survival after X-ray irradiation was examined in the X-ray resistant, X-ray sensitive and "wild type" strains. The survival curves are given in Figure 31. The X-ray resistant strains N123-P4 and 10018-P10 show identical survivals after irradiation. This is not surprising as they are allelic mutations. The survival of the Xᵣ strains was much greater than that of the "wild type" particularly at lower doses. At higher doses, the difference in survival was less extreme. For example, at 25KR the survival of the Xᵣ strains was 88% compared to 13% for the
Figure 30. U.V. Dose vs. Survival of Haploid *S. cerevisiae*: "Wild Type" vs. a U.V. Sensitive Strain.
Figure 31. X-ray Dose vs. Survival of Haploid S. cerevisiae: A Comparison of Strains with Different X-ray Sensitivities.
"wild type", a factor of six while at 100 KR, the difference was only a factor of two.

Strain 842-3A was very X-ray sensitive. At 20 KR the survival of the X-ray sensitive strain was 2% compared to 18% in the "wild type" strain.

Spontaneous Lethal Sectoring:--Five different strains of yeast were examined by pedigree analysis. The colony forming ability of individual cells was analysed. Four types of events were found and they were classified as follows:

1. Normal:--In these pedigrees all products survived to produce macroscopic colonies.
2. Lethal Sectors:--Lethal sectors were said to have occurred when a pedigree product failed to produce a macroscopic colony. The original cell isolated was classed as a 'survivor' as one part of the pedigree did produce a macroscopic colony.
3. Abortive:--A pedigree was classed as abortive if the cell originally isolated underwent a few residual divisions but failed to produce a macroscopic colony. These were classed as non-survivors since no macroscopic colonies were produced.
4. Dead:--Cells were classified as dead when they did not undergo even one cell division after isolation.

The results of pedigree analysis, to the two cell stage, is
given in Table VII. The data is based upon several experiments with each strain.

The frequency of spontaneous lethal sectoring in the "wild type" strain, M21-3D, was 0.38%. The only strain that differed significantly in the amount of spontaneous lethal sectoring from the "wild type", was strain 842-3A, the X-ray sensitive and recombination deficient strain. This strain showed a frequency of spontaneous lethal sectoring of 3.68%, an increase of a factor of 9.7 over the "wild type" strain. The frequencies of spontaneous lethal sectoring in all the strains studied are compared by ratios in Table VIII.

The possibility that handling the cells could give rise to the differences in the amount of spontaneous lethal sectoring in different strains was tested by comparing the frequencies of deaths, abortives and spontaneous lethal sectors in each strain. The results are given in Table IX. No correlation was found between spontaneous lethal sectors and cell deaths. Thus, variation in lethal sectoring frequencies were not due to a strain's ability to survive handling.

It was observed, however, that lethal sectoring and abortive frequencies were not independent (0.001<p<0.01). Those strains showing high abortive frequencies were higher in their lethal sectoring frequencies.
<table>
<thead>
<tr>
<th>Strain</th>
<th>M21-3D</th>
<th>N123-P4</th>
<th>10018-P10</th>
<th>842-3A</th>
<th>277-1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>&quot;wild type&quot;</td>
<td>X-Ray Resistant</td>
<td>X-Ray Resistant</td>
<td>X-Ray Sensitive</td>
<td>U.V. Sensitive</td>
</tr>
<tr>
<td>No. of Cells Isolated</td>
<td>538</td>
<td>280</td>
<td>296</td>
<td>241</td>
<td>399</td>
</tr>
<tr>
<td>No. of Non-Surviving Cells</td>
<td>10</td>
<td>12</td>
<td>3</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>%</td>
<td>1.9%</td>
<td>4.3%</td>
<td>1.0%</td>
<td>10.0%</td>
<td>6.0%</td>
</tr>
<tr>
<td>No. Dead</td>
<td>8</td>
<td>11</td>
<td>3</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>%</td>
<td>1.5%</td>
<td>3.9%</td>
<td>1.0%</td>
<td>4.2%</td>
<td>2.8%</td>
</tr>
<tr>
<td>No. Abortive</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.0%</td>
<td>5.8%</td>
<td>3.2%</td>
</tr>
<tr>
<td>No. of Surviving Cells</td>
<td>528</td>
<td>268</td>
<td>293</td>
<td>217</td>
<td>375</td>
</tr>
<tr>
<td>No. Not Separated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>No. Separated and Surviving</td>
<td>522</td>
<td>263</td>
<td>270</td>
<td>217</td>
<td>362</td>
</tr>
<tr>
<td>Expected No. of Colonies&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1044</td>
<td>526</td>
<td>540</td>
<td>434</td>
<td>724</td>
</tr>
<tr>
<td>No. of Colonies</td>
<td>1040</td>
<td>526</td>
<td>539</td>
<td>418</td>
<td>718</td>
</tr>
<tr>
<td>No. Lethal Sectors</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>% Lethal Sectors</td>
<td>0.38%</td>
<td>0.0%</td>
<td>0.19%</td>
<td>3.68%</td>
<td>0.83%</td>
</tr>
</tbody>
</table>

Table VII. Spontaneous lethal sectoring in haploid *S. cerevisiae*: A comparison of strains that differ in their ability to survive U.V. or X-ray irradiation.

<sup>a</sup> These cells were lost to the experiment due to technical difficulties.

<sup>b</sup> The number of colonies expected if both pedigree products produced macroscopic colonies.
**Table VIII.** Spontaneous lethal sectoring in haploid *S. cerevisiae*: A comparison of normal and special strains by ratios. \(^a\)All fractions (a/b) represent the number of lethal sectors which were found divided by the number of colonies possible if all separated cells had produced macroscopic colonies. This ratio is from the "wild type" haploid, M21-3D. \(^*\) These values were obtained by Chi\(^2\) with Yate's correction for continuity.

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>TYPE</th>
<th>SPECIAL STRAIN(^a)</th>
<th>NORMAL STRAIN(^b)</th>
<th>RATIO-SPECIAL/ NORMAL</th>
<th>CHI SQ.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a/b</td>
<td>c/d</td>
<td>ad/bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N123-P4</td>
<td>X-ray</td>
<td>0.526</td>
<td>4/1044</td>
<td>0.00</td>
<td>0.79*</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10018-P10</td>
<td>X-ray</td>
<td>1/540</td>
<td>4/1044</td>
<td>0.48</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>842-3A</td>
<td>X-ray</td>
<td>16/434</td>
<td>4/1044</td>
<td>9.70</td>
<td>24.05*</td>
<td>6 X 10^-7</td>
</tr>
<tr>
<td></td>
<td>Rec(^-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>6/724</td>
<td>4/1044</td>
<td>2.15</td>
<td>0.82*</td>
<td>0.35</td>
</tr>
<tr>
<td>STRAIN</td>
<td>M21-3D</td>
<td>N123-P4</td>
<td>227-1b</td>
<td>777-4D</td>
<td>842-3A</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>DEATHS</td>
<td>1.50%</td>
<td>3.90%</td>
<td>2.80%</td>
<td>1.00%</td>
<td>4.20%</td>
<td></td>
</tr>
<tr>
<td>ABORTIVES</td>
<td>0.40%</td>
<td>0.40%</td>
<td>3.20%</td>
<td>0.00%</td>
<td>5.80%</td>
<td></td>
</tr>
<tr>
<td>LETHAL-SECTORS</td>
<td>0.38%</td>
<td>0.00%</td>
<td>0.83%</td>
<td>0.19%</td>
<td>3.68%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COMPARISON</th>
<th>SLOPE</th>
<th>t-VALUE</th>
<th>PROBABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEATH vs. ABORTIVE</td>
<td>0.3686</td>
<td>1.0799</td>
<td>0.300&lt;p&lt;0.500</td>
</tr>
<tr>
<td>DEATH vs. LETHAL-SECTORING</td>
<td>0.5324</td>
<td>0.3563</td>
<td>0.700&lt;p&lt;0.800</td>
</tr>
<tr>
<td>ABORTIVE vs. LETHAL-SECTORING</td>
<td>0.5678</td>
<td>5.9580</td>
<td>0.001&lt;p&lt;0.010</td>
</tr>
</tbody>
</table>

Table IX. Correlations between the frequencies of death, abortives and lethal sectors in different strains of *S. cerevisiae*. 
X-ray Induced Lethal Sectoring:—Lethal sectoring after X-ray irradiation was examined in three strains of \textit{S. cerevisiae}. A dose of 2 KR was given to the X-ray sensitive strain, 842-3A, the X-ray resistant strain, N123-P10, and the "wild type" strain, M21-3D. Table X gives the results for this series of experiments.

There were no significant changes in lethal sectoring frequencies after irradiation, which confirmed that X-rays do not induce lethal sectoring in haploid Saccharomyces. Strain 842-3A, the X-ray sensitive mutant, maintained the same high level of lethal sectoring as it had in the unirradiated samples.

U.V. Induced Lethal Sectors:—The effect of U.V. upon lethal sectoring was examined in the "wild type" strain, M21-3D, and the U.V. sensitive strain, 227-1b. It was found that U.V. induced lethal sectoring in these haploid strains (Table XI). The frequency of lethal sectoring appears to increase with dose. In the "wild type" strain doses of 250 and 450 ergs/mm.\(^2\) produced 1.0\% and 2.3\% lethal sectoring compared with 0.38\% in the control.

The U.V. sensitive and "wild type" strains were compared at doses which produced the same survival. A dose of 25 ergs/mm.\(^2\) given to the U.V. sensitive strain gave a survival of 67\%. This was considered comparable to a survival of 69.5\% found in the "wild type" strain after a dose of 450 ergs/mm.\(^2\). It was found
<table>
<thead>
<tr>
<th>Strains</th>
<th>M21-3D</th>
<th>N123-P4</th>
<th>842-3A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>&quot;wild type&quot;</td>
<td>x&lt;sup&gt;r&lt;/sup&gt;</td>
<td>x&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of Cells Isolated</td>
<td>103</td>
<td>360</td>
<td>601</td>
</tr>
<tr>
<td>No. of Non-Surviving Cells</td>
<td>34</td>
<td>35</td>
<td>274</td>
</tr>
<tr>
<td>%</td>
<td>33%</td>
<td>9.7%</td>
<td>45.6%</td>
</tr>
<tr>
<td>No. of Surviving Cells</td>
<td>69</td>
<td>325</td>
<td>327</td>
</tr>
<tr>
<td>No. Not Separated</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>No. Separated</td>
<td>68</td>
<td>317</td>
<td>326</td>
</tr>
<tr>
<td>No. Colonies Expected</td>
<td>136</td>
<td>634</td>
<td>652</td>
</tr>
<tr>
<td>No. Colonies Seen</td>
<td>136</td>
<td>633</td>
<td>629</td>
</tr>
<tr>
<td>No. of Lethal Sectors</td>
<td>0</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>% Lethal Sectoring</td>
<td>0.0%</td>
<td>0.29%</td>
<td>3.83%</td>
</tr>
<tr>
<td>% Spontaneous Lethal Sectoring</td>
<td>0.38%</td>
<td>0.0%</td>
<td>3.68%</td>
</tr>
<tr>
<td>Net Change</td>
<td>-0.38%</td>
<td>+0.29%</td>
<td>+0.15%</td>
</tr>
</tbody>
</table>

Table X. X-ray induced Lethal Sectoring in Strains of *S. cerevisiae*: A Comparison of Normal, X-Ray Resistant and X-Ray Sensitive Strains.
<table>
<thead>
<tr>
<th>Strain</th>
<th>M21-3D</th>
<th>M21-3D</th>
<th>227-lb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>&quot;wild type&quot;</td>
<td>&quot;wild type&quot;</td>
<td>uvs-9</td>
</tr>
<tr>
<td>Dose</td>
<td>250 ergs/mm.²</td>
<td>450 ergs/mm.²</td>
<td>25 ergs/mm.²</td>
</tr>
<tr>
<td>No. Cells Isolated</td>
<td>230</td>
<td>203</td>
<td>480</td>
</tr>
<tr>
<td>No. of Non-Survivors</td>
<td>26</td>
<td>62</td>
<td>158</td>
</tr>
<tr>
<td>%</td>
<td>11.3%</td>
<td>30.5%</td>
<td>32.9%</td>
</tr>
<tr>
<td>No. of Surviving Cells</td>
<td>204</td>
<td>141</td>
<td>322</td>
</tr>
<tr>
<td>No. Not Separated</td>
<td>10</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>No. Separated</td>
<td>194</td>
<td>133</td>
<td>296</td>
</tr>
<tr>
<td>No. Colonies Expected</td>
<td>388</td>
<td>266</td>
<td>592</td>
</tr>
<tr>
<td>No. Colonies Observed</td>
<td>384</td>
<td>260</td>
<td>542</td>
</tr>
<tr>
<td>No. of Lethal Sectors</td>
<td>4</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>% Lethal Sectors</td>
<td>1.0%</td>
<td>2.3%</td>
<td>8.4%</td>
</tr>
<tr>
<td>% of Spontaneous Lethal Sectoring</td>
<td>0.38%</td>
<td>0.38%</td>
<td>0.83%</td>
</tr>
<tr>
<td>Net Change</td>
<td>+0.62%</td>
<td>+0.92%</td>
<td>+7.57%</td>
</tr>
<tr>
<td>Probability that Induced L.S. equals Spontaneous L.S.</td>
<td>0.15</td>
<td>0.002</td>
<td>$2 \times 10^{-12}$</td>
</tr>
</tbody>
</table>

Table XI. U.V. Induced Lethal Sectoring in S. cerevisiae: A Comparison of a U.V. Sensitive and "wild type" Strain.
that at comparable survivals the U.V. sensitive strain produced more lethal sectors (8.4%) than the "wild type" strain (2.3%). This difference is highly significant (p≈10^{-3}).
DISCUSSION

I. The Results of Pedigree Analysis

Pedigree analysis of U.V. induced mitotic recombination revealed that recombinant pedigree products could segregate in both the first and second post-irradiation generations. A total of 22 cases of recombinant chromosome arms were examined in this study. Of these, 18 occurred exclusively in the first generation after irradiation. These pedigrees were all classed as Type I segregants and involved exclusively reciprocal exchanges between homologous chromatids. The four recombinants which segregated in the second post-irradiation were more difficult to explain.

The Mechanism of First Generation Segregation:-Pedigrees (5, 74, 111, 112 and 133) demonstrated a single recombinant event in Fragment 1. In pedigrees 111 and 112 the cross-over occurred between the centromere and the Ac\(_4^r\) locus, producing segregants that were auxotrophic for adenine and histidine and resistant to actidione as well as segregants which were prototrophic and actidione sensitive. The simplest mechanism to for this event is illustrated in Figure 32. The segregants in pedigree 5 can be accounted for by a reciprocal cross-over between the Ac\(_4^r\) and the ad\(_4^2\) markers (Figure 33).

Recombinant pedigree 133 (Figure 26) may have resulted from a cross-over in either the centromere to Ac\(_4^r\) region or the Ac\(_4^r\) to
Mitotic Recombination

Proposed Mechanism

Pedigrees 111 and 112

Figure 32.
Mitotic Recombination

Proposed Mechanism

Pedigree 5

Figure 33.
the ad$_2$ region. Since three of the four cell lines were lost as lethal sectors and because no viable spores were obtained from the surviving sector, the exact position of the cross-over is unknown. It is also possible that the auxotrophic portion of this pedigree segregated in the second generation. Pedigree 74 (Figure 19) was similar to pedigree 133 as it also contained a primary lethal sector, produced no viable spores and could have been the result of a recombination in either of the chromosome regions discussed above.

Single recombination events were detected in pedigrees 40, 41, 45, 98 and 121. Pedigree 45 was the only case where the cross-over event occurred between the centromere and the thr$_3$ marker (Figure 34). The crossing-over in the other pedigrees occurred in the thr$_3$-is$_1$ region (Figure 35).

Pedigree 146 (Figure 28) could not be explained by a single cross-over event. It was concluded that cross-overs involving all four strands were necessary to obtain the recombinant products found by pedigree analysis. The proposed mechanism is illustrated in Figure 36.

Pedigree 107 had a primary lethal sector. The surviving cell line required valine, and tetrad analysis revealed that the survivors were homozygous for the "wild type" alleles of the ad$_2$ and hi$_8$ loci. It was postulated that there had been a
Mitotic Recombination

Proposed Mechanism

Pedigree 45

Figure 34.
Mitotic Recombination

Pedigrees 40, 41, 98 and 121.

Figure 35.
Mitotic Recombination

Proposed Mechanism

Pedigree 146

Figure 36.
cross-over in two chromosome arms and that reciprocal products were lost in the lethal sector (Figure 37).

Pedigree 138 had cross-overs in three different chromosome arms in such a way that all pedigree products were recombinant. All recombinant products segregated out in the first generation after irradiation. The proposed mechanism is illustrated in Figure 38.

The Mechanism of Second Generation Segregation:-The segregation of recombinant products in the second post-irradiation generation was found in four cases. The segregation could be best explained by the segregation of heterologous DNA (Figure 6).

Two of these pedigrees, numbers 15 and 71 involved the segregation of markers only in the second generation. In pedigree 15 (Figure 12), a pair of linked markers, is₁ and tr₂, segregated in the second generation in cell lines on both sides of the pedigree. This can be accounted for by a number of mechanisms: simultaneous cross-overs in both first post-irradiation generation division products; non-disjunction and two cross-overs; or a modification of Type II segregation where cross-overs may involve four single strands of DNA. There was no evidence in any other pedigree either for a second generation reciprocal recombination or non-disjunction (particularly, where two cross-overs are also required). It, therefore, seems unlikely that either of these two account for the segregation pattern found in this pedigree. A double single-strand DNA exchange
Mitotic Recombination

Proposed Mechanism

**CHROMOSOME III**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>val</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**FRAGMENT 1**

<table>
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<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Ac^r$</td>
<td>ad</td>
<td>hi</td>
<td>+</td>
</tr>
</tbody>
</table>

**CROSS-OVER**

1 AND 3

2 AND 4

1 AND 3

**SEgregation**

1 AND 3

-lost in primary lethal-sector

Figure 37.
Mitotic Recombination

Proposed Mechanism

Pedigree 138

Figure 38.
Involving different double stranded DNA helices could explain pedigree 15. The mechanism is illustrated in Figure 39.

Although the recombinant segregation pattern for the valine locus is obscured in pedigree 71, because of the first post-irradiation division segregation of the unlinked marker, is1, this pedigree is not easily explained as a Type II segregation pattern since it involves a non-reciprocal second generation segregation.

Pedigrees 54 and 73 display segregation of linked markers segregating in both the first and second post-irradiation generation. These segregations cannot be explained by conventional recombination mechanisms. Pedigrees 54 and 73 demonstrate conclusively that the delayed segregations were the result of events which occurred one segregation earlier.

The segregation pattern in pedigree 54 can be accounted for by a Type II segregation where three reciprocal single-stranded DNA exchanges occur (Figure 40). Pedigree 73 required two single-strand cross-overs and two double-stranded double cross-overs. This is illustrated in Figure 41.

The segregation of unrepaired heterologous hybrid DNA can account for the four pedigrees demonstrating the delayed segregation of genetic markers. This model is particularly useful in explaining the segregation of linked markers in two consecutive gener-
Mitotic Recombination

Proposed Mechanism

Pedigree 15

Figure 39.
Mitotic Recombination

Proposed Mechanism

Pedigree 54

Figure 40.
Mitotic Recombination

Proposed Mechanism

Pedigree 73

Figure 41.
ations such as described by Wood (1967), Haefner (1966) and in this study. These events can be considered "non-reciprocal recombination without gene-conversion".

An Examination of Other Pedigree Experiments

Haefner (1966) reported that pedigrees from U.V.-irradiated S. cerevisiae always show reciprocal mitotic recombination and that recombination was not restricted to the first post-irradiation generation. However, a critical analysis of his recombinant pedigrees reveals that the results Haefner obtained were not strictly reciprocal. In fact, in the six pedigrees presented, none of them show simple reciprocal recombination such as found in 18 of the 22 recombinant chromosome arms in this study. Haefner's six recombinant pedigrees are diagrammed in Figure 42.

In recombinant pedigree A the lysine auxotrophs appear on both sides of the pedigree. Unfortunately, the cell lines from pedigree analysis were not subjected to genetic analysis, but it is evident that either mutation or an unconventional event such as accounted for by Type II segregations occurred.

Among the viable products of pedigree B are only lysine auxotrophs. There were 16 viable cell lines and their arrangement makes it unlikely that even the non-reciprocal recombination without conversion predicted by Type II segregation could account for this pattern. It is more likely that either gene-
Figure 42. U.V. Induced Recombination: Pedigrees from Haefner (1966). Symbols: v = viable; a = dead; 0 = abortive; / = a lethal sector.
conversion or mutation occurred in this pedigree.

Haefner's third pedigree, C, corresponds exactly to the phenotype segregation expected from a Type II event, while his fourth pedigree, D, can be accounted for by two consecutive Type II segregations. However, in pedigree D, gene-conversion or mutation is necessary to account for the 3:1 auxotroph to prototroph ratio in the fifth post-irradiation generation. It is also possible that the U.V. induced a replicating instability of the original first division cell.

A series of reciprocal recombinations in several post-irradiation generations can account for pedigree E. Pedigree E, could also be explained by a Type II event in the second post-irradiation generation or a mixture of Type I and Type II events.

Pedigree F can be explained either by a Type II segregation followed much later by a single reciprocal event on both sides of the pedigree.

In summary, Haefner's pedigrees demonstrate that U.V. induced mitotic recombination involves complex segregations, which are not necessarily reciprocal. The segregation of the uncorrected hybrid DNA is a useful model in accounting for these segregation patterns.

Spontaneous Recombination:—Hastie (1968) has studied spontaneous mitotic recombination in *Verticillium albo-atrum*. The technique used was the following: phialides are branches of fungal
Conidiophores on which asexual spores (conidia) are formed. Phialides are initially uninucleate and mitosis is followed by the migration of one of the daughter nuclei into the developing conidium. This conidium is abstracted before the daughter nucleus remaining in the phialide divides a second time. The free conidium is taken by micromanipulation and as the process is repeated each phialide produces a phialide family.

Figure 43. The Origins of a Phialide Family Pedigree.

Hastie studied the phialide products in over 600 families using a diploid that was heterozygous at five loci. Out of these 600 families 108 showed segregation for at least one locus. Progeny testing of the conidia in 42 segregating families was undertaken and 47 segregations were recorded. Hastie made the following conclusions: (1) recombination in families where all conidia were viable was always reciprocal (2) no evidence for a delay between mitotic crossing-over and marker segregation was found.
In summary, three studies of individual mitotic segregations have been reported. This study reports that in four cases out of 22 recombinant chromosome arms, there was a delay between mitotic crossing-over and marker segregation. Haefner (1966) has published six pedigrees obtained by pedigree analysis of U.V. irradiated S. cerevisiae. In all six pedigrees delayed segregation is evident. On the other hand, Hastie in his phialide analysis of spontaneous mitotic recombination in Verticillium found no evidence at all for delayed segregation. This brings up two questions: (1) what are the differences between the methods used in this study and those used by Haefner and would these differences, if any, account for the relative predominance of delayed segregation found by Haefner (2) are spontaneous and U.V. induced mitotic recombination dependant upon the same mechanism?

(1) A comparison of techniques used in this study with those of Haefner can probably account for the differences in results. Haefner treated stationary phase cells with a U.V. dose of 910 ergs/mm.$^2$ and then undertook pedigree analysis. In this study early stationary phase cells were used and a U.V. dose of 1500 ergs/mm.$^2$ was followed by optimal photoreactivation with visible light.

It can be proposed that the unrepaired thymine dimers which are in excess of the cell's repair capability in the non-photo-
reactivated samples are maintained and are responsible for the delayed segregation.

(2) If this conclusion is reached then it becomes necessary to consider if U.V. induced mitotic recombinations are similar. Three studies have been reviewed in this discussion. They show that delayed segregation may be a consequence of the U.V. treatment.

The specific treatment, however, is important in determining the type of recombinant event. In this study it was seen that U.V. induced a majority (18/22) of "spontaneous-like" events while a treatment that excluded photoreactivation (Haefner 1966) lacks these events. It can be concluded that U.V. can induce a pattern of recombinant segregation that does not appear to occur spontaneously.

The "Uncorrected" Hybrid DNA Recombination Model

The study of U.V. induced intergenic mitotic recombination has produced evidence that the widely accepted mechanism of whole chromatid (i.e. a single Watson-Crick double helix) exchange as proposed by Stern (1936) is inadequate. Mitotic recombination involving sub-chromatid or single-stranded DNA exchange would result in a delay between the occurrence of the cross-over and the segregation of the loci. This delay was detected in four cases, and to explain these results a model has
been adopted (Wood 1967).

This model, as already discussed, involves the segregation of long regions of uncorrected heterologous hybrid DNA. The existence of heterologous DNA implies that correction is possible which would lead to gene-conversion. The Whitehouse (1965a) and Holliday (1964) models of recombination propose that "short regions" of the DNA become hybrid. The data presented in this study do not fit these "hybrid" repair recombination models.

The Extent of Hybrid Regions:--The molecular complexities of exchanging long regions of single-stranded DNA are immense. There is, however, a precedent for the exchange of "long regions" of single-stranded DNA.

Esposito (1968b) found that in yeast U.V. induced gene-conversion could occur simultaneously for three linked loci. She found that in three out of fourteen cases of U.V. induced mitotic recombination, the prototrophic sectors were still heterozygous for the three linked loci. The linkage groups and her results are presented in Figure 44.

It should be pointed out that Esposito's results need not be accounted for by gene-conversion. Since in plating experiments, all products of a recombination event may not be recovered, a Type II segregation after a single single-stranded DNA exchange would result in an auxotrophic sector along with heterozygous prototrophs.
Loci Examined
(Chromosome II)

Genetic Analysis of Sectored Colonies:

<table>
<thead>
<tr>
<th>Class</th>
<th>Auxotrophic Sector</th>
<th>Prototrophic Sector</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reciprocal</td>
<td>$\text{ly}_2 \text{ ty}_1 \text{ hi}_7$</td>
<td>$+ + +$</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$\text{ly}_2 \text{ ty}_1 \text{ hi}_7$</td>
<td>$+ + +$</td>
<td></td>
</tr>
<tr>
<td>Non-Reciprocal</td>
<td>$\text{ly}_2 \text{ ty}_1 \text{ hi}_7$</td>
<td>$+ + +$</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 44. Results of U.V. induced mitotic recombination in *S. cerevisiae* Esposito (1968). The meiotic map for the loci studies is given. The symbols are: ly=lysine, ty=tyrosine, hi=histidine. The numbers in parenthesis are meiotic map units.

The apparent simultaneous "conversion" of linked loci has not been found in studies of spontaneous meiotic recombination in Neurospora (Stadler and Towe 1968). However, Strickland (1958) has reported a single case in Aspergillus where conversion occurred simultaneous for two linked loci, pro$_1$ and paba$_1$, which are separated by at least nine meiotic map units. This unique report of the simultaneous conversion of two linked loci given an indication that in rare cases, lengthy regions of hybrid DNA may form spontaneously.

Direct evidence concerning the incorporation of single-strand DNA can be obtained from experiments in bacterial transformation.
It has been shown that the recombination process during bacterial transformation involves only one of the DNA strands of the donor double helix into the recipient chromosome (Bresler, Kreneva, Kushev and Moesevitskii 1964; Fox 1966 and others).

Bresler and his co-authors (Bresler, Kreneva and Kushev 1968) have examined the coincidence of the correction of hybrid heterozygotes formed during transformation. The simultaneous repair of the heterozygous DNA gives direct evidence about how much transforming DNA can be handled by a single event. Bresler et al made the discovery that simultaneous repair encloses long regions of the DNA. The regions are in the order of $5-6 \times 10^6$ daltons or $10^4$ nucleotide pairs. Since $10^4$ nucleotides correspond to 1000 turns of the Watson-Crick double-helix, it is remarkable that this size single-stranded DNA can be incorporated. It should be noted that this is a minimum estimate as much larger strands may be incorporated in a single event but be beyond simultaneous repair.

An estimate can be made of the length of single-stranded DNA which needs to be exchanged to account for the results obtained in this study. The loci exchanged span the region distal to the cross-over and may involve between 1 and 5% of the haploid content which has been estimated to be $2.2 \times 10^7$ nucleotides (Ogur, Minckler, Lindegren and Lindegren 1952). The exchanged subunits would then correspond to between $1 \times 10^5$ and $5 \times 10^5$ nucleotides.
An Hypothesis Concerning the Mechanisms of Recombination

The following hypothesis is proposed to explain the data in this study and other studies which have been reviewed in this report.

Hypothesis: Damage in the cell caused by U.V. irradiation can be repaired by two systems, "excision" repair and "post-replication" repair. Usually U.V. damage is corrected by "excision" repair but under conditions where the damage is beyond the cell's "excision" repair capability, "post-replication" repair corrects the excess damage.

On the other hand, "post-replication" repair is the major repair system for damage from X-ray irradiation. A minor portion of the damage can be corrected by "excision" repair.

This model, then, has two separate pathways, connected by a shunt, for the correction of damage from U.V. and X-ray irradiation. This is a modification of an earlier model proposed by Resnick (1968).

"Excision" repair favours reciprocal mitotic recombination, involving the exchange of whole chromatids (Stern 1936) or one DNA double helix. "Post-replication" repair, however, results in the exchange of single-stranded DNA and the formation of hybrid DNA. The correction, or partial correction, of heterologous hybrid DNA results in gene conversion, or non-reciprocal recombination, while the uncorrected heterologous DNA results in delayed segregations.
The Implications of this Hypothesis:—This model accounts for a number of phenomenon such as (1) the difference in response of inter- and intragenic recombination to temperature, photoreactivation liquid holding recovery and fluorodeoxyuridine treatment (2) fine mapping in yeast with X-rays (3) the coincidence of outside marker crossing-over with conversion (4) the influence that radiation sensitive mutants have upon recombination.

(1) Since this hypothesis accounts for inter- and intragenic recombination by different mechanisms, it follows that different treatments will have different effects upon each phenomenon. For example, it has been shown that photoreactivation with visible light after U.V. irradiation drastically reduces non-reciprocal intragenic recombination while having little effect upon reciprocal intergenic recombination (Pittman 1961; Parry and Cox 1965, 1968a). Photoreactivation must result in the removal of the "excess thymine dimers which would have required "post-replication" repair, reducing the amount of intragenic recombination.

This hypothesis can also account for the fact that liquid holding recovery greatly increases intragenic recombination while reducing intergenic recombination (Parry and Cox 1968a). According to this hypothesis "post-replication" repair rather than "excision" repair must be favoured by liquid holding recovery. The following evidence indicates the importance of "post-replication" repair in liquid holding recovery: (1) rec strains of E. coli which have lost the recombination ability required for
"post-replication" repair do not demonstrate liquid holding recovery (Ganesan and Smith 1968) (2) "excision" repair requires an energy source (Setlow 1965) and liquid holding recovery is carried out in the absence of an energy source. The consequence of liquid holding recovery favouring "post-replication" repair is that during liquid holding recovery non-reciprocal intragenic recombination is also favoured.

It has been found that in Aspergillus, fluorodeoxyuridine which inhibits DNA synthesis induces intergenic but not intragenic recombination (Beccari, Modigliani and Morpurgo 1967). Esposito (1968b) demonstrated that fluorodeoxyuridine inhibits U.V. induced mitotic recombination. It is a likely consequence of the proposed hypothesis, that in the presence of an energy source, fluorodeoxyuridine or any other inhibitor of DNA synthesis would result in decreased "post-replication" repair producing increased intergenic recombination and reduced intragenic recombination.

(2) This hypothesis explains the basis of intragenic fine structure mapping as described by Manney and Mortimer (1964). They found that X-ray induced simultaneous gene conversion of two intragenic markers with a frequency inversely proportional to the distance between the markers. X-ray maps, so produced, were free from map expansion which is a problem in any other mapping system. This model explains fine structure mapping in terms of the amount of heterologous DNA which can be repaired in a single event.

It can be shown that the convertants obtained by X-ray
mapping were the results of the same repair event. For example, Esposito (1968a) found that all convertants of heteroallelic crosses, which were in the cis-trans position produced ratios of conversions of proximal vs. distal of 1:3 and 3:1 and 3:1 and 1:3 and lacked any ratios which would have indicated two separate repair events. It can then be concluded that single repair events are the cause of simultaneous conversions. The occurrence of these simultaneous conversions will be inversely proportional to the distance between the alleles and, therefore, produce a linear map free from expansion.

(3) The coincidence of crossing-over of outside markers with conversion can be explained by this hypothesis. Despite the differences between the "post-replication" repair and "excision" repair systems both processes require non-conservative repair replication to patch single-strand gaps. If non-conservative repair replication is the recombinogenic event, damage corrected by "post-replication" repair would be prone to reciprocal crossing-over involving the outside markers.

(4) This model has also an advantage over other recombination because it can account for the properties of a number of U.V. and X-ray sensitive mutants which have been isolated in Ustilago (Holliday 1965, 1967) and in Saccharomyces (Snow 1967, 1968; Nakai and Matsumoto 1967).

It can be predicted from the model presented that in U.V. sensitive strains which lack excision repair, and thus are
not X-ray sensitive, all U.V. damage would be in excess of the cell's "excision" repair capability and "post-replication" repair would be greatly increased. These strains would be expected to show a greater than normal increase in U.V. induced gene conversion. The uvs-3 mutant of Ustilago (Holliday 1967) and the uvs-4 and uvs-9 mutants of Saccharomyces (Snow 1967, 1968) show increased gene conversion among survivors after U.V. irradiation and are U.V. sensitive but not X-ray sensitive.

It should also be possible to isolate mutants of the shunt between the U.V. and X-ray repair pathways which would be U.V. sensitive and moderately X-ray sensitive and show increased intergenic recombination. Holliday (1967) and Snow (1968) have described such mutants.

Moreover, an X-ray sensitive mutant $X_1^S$ has been isolated in Saccharomyces (Nakai and Matsumoto 1967). When the $X_1^S$ mutant is homozygous in a diploid strain, the strain is recombination deficient since the mutation prevents X-ray induced intragenic mitotic recombination, but has no effect upon the frequency of spontaneous recombination (Nakai personal communication). If there are separate pathways for intergenic and intragenic recombination, then the $X_1^S$ mutant may allow U.V. induced intergenic recombination to occur. If this is found to be the case, then this would be excellent corroborative evidence for the recombination mechanisms proposed above.
Other Models:- The possibility that a "copy choice" mechanism (e.g. Freese 1957) is responsible for the delayed segregation must be considered.

One of the main objections to "copy choice" theories is that unless the "breakage and rejoining of sister strands" is also hypothesized, "copy choice" would be restricted to newly synthesized strands. In the four cases of delayed segregation found in this study, none of the strand exchanges need occur in any but the newly synthesized DNA strands. However, four cases of delayed segregation can not be seriously considered as excluding the possibility that "old" strands may be exchanged.

However, the theories of "copy choice" mechanisms have been largely discounted (for review see Fincham and Day 1965; Whitehouse 1965b); "copy choice" will not be further examined.

The models of recombination so far discussed have been based upon the assumption that a single chromatid is equivalent to one Watson-Crick double-stranded DNA helix. The prime reason for making this assumption is that mutations occur at the half-chromatid (i.e. single-stranded DNA) and/or whole-chromatid (i.e. double-stranded DNA) level (Nasim and Auerbach 1967). Further evidence which would help identify a chromatid as a single double helix is the replication pattern found by autoradiographic techniques (Cairns 1963; Taylor 1957). Although there is much evidence that higher organisms have multi-stranded
chromosomes (e.g. LaCour and Pelc 1959), the chromosomes of the fungi are very small and probably much simpler.

The possibility that the chromosomes had already replicated at the time of irradiation can be excluded because it has been shown that DNA synthesis begins as the bud forms (Williamson 1965), and during these experiments only non-budding cells were selected by micromanipulation.

**Lethal Sectoring and Recombination**

Pedigree analysis reveals the fate of individual cells and gives information about the pedigree products' colony forming ability. A lethal sector is said to occur when a cell-line from a pedigree fails to produce a macroscopic colony.

**The Influence of Lethal Sectoring Upon Recombination:**

Radiation induced mitotic recombination of a single genetic locus often results in colonies which are wholly prototrophic. Yamasaki et al (Yamasaki, Ito and Matsudaira 1964) considered the "pure" colonies produced by X-ray induced mitotic recombination to be the result of non-reciprocal recombination. In this study the existence of "pure" prototrophic products was confirmed during pedigree analysis. It was found, however, that "pure" prototrophic colonies occurred only where the "wild type" had been removed from the progeny by a primary lethal sector.

Haefner (1966) has proposed that all "pure" recombinant colonies
resulting from mitotic recombination are the result of primary lethal sectors. The data presented in this study supports this view.

The Effect of Photoreactivation on Lethal Sectoring:- The effect of photoreactivation upon lethal sectoring was not directly examined in this study. However, if the premise that "pure" prototrophic colonies are due only to lethal sectors, is accepted, then the proportion of "pure" prototrophic colonies that appear in plating experiments gives an estimate of the relative amounts of lethal sectoring which occurred in plating experiments in photoreactivated and non-photoreactivated samples.

From the results given in Table II it is obvious that photoreactivation reduces lethal sectoring. The reasoning is as follows: the "pure" prototrophic colonies in the non-photoreactivated samples account for more than 50% of the total recombinants while in the photoreactivated sample the "pure" prototrophic recombinants only account for 10% of the total. Although these figures themselves are biased because the recombinant detection is altered by the lethal sectors, it becomes clear that the photoreactivated samples contain far fewer lethal sectors.

The Effect of Lethal Sectoring on Recombination:- The lethal sectoring is as likely to produce a "pure" auxotrophic colony as a "pure" prototrophic colony, it can therefore be seen that the
amount of lethal sectoring will bias the recombination frequencies. In this light the results previously presented in Table II can be reconsidered (Table XII).

<table>
<thead>
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<th>1500 ergs/mm.²</th>
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</thead>
<tbody>
<tr>
<td>Photoreactivation</td>
<td>none</td>
<td>7.5 minutes</td>
</tr>
<tr>
<td>Survival</td>
<td>12%</td>
<td>45%</td>
</tr>
<tr>
<td>No. Colonies/Examined</td>
<td>2087</td>
<td>2998</td>
</tr>
<tr>
<td>No. &quot;Pure&quot; Red Recombinants</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>No. &quot;Pure&quot; White Recombinants (Estimate)</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>No. Mosiac Recombinants</td>
<td>46</td>
<td>129</td>
</tr>
<tr>
<td>Adjusted Total</td>
<td>142</td>
<td>159</td>
</tr>
<tr>
<td>Adjusted % Recombination</td>
<td>6.8%</td>
<td>5.3%</td>
</tr>
</tbody>
</table>

Table XII. Recombination frequencies adjusted for lethal sectoring (data from plating experiments).

When the data are adjusted for the effect of lethal sectoring, it can be seen that photoreactivation reduces recombination by more than 20%.

Parry and Cox (1965) studied the effect of photoreactivation upon inter and intragenic mitotic recombination in yeast. They found that photoreactivation had no effect upon the frequency of intergenic recombination while it greatly reduced intragenic recombination. Considering the effect which photoreactivation
had upon intergenic recombination in this study, it is likely that their intergenic recombination is also lowered by photo-reactivation but is masked by the reduction of lethal sectoring. Since the intragenic recombination is primarily non-reciprocal, lethal sectoring has no effect upon the efficiency of detecting these non-reciprocal events.

This type of analysis should be applied to all data on mutation and recombination, particularly, when examining strains with altered radiosensitivity or recombination ability, as these strains may display abnormal lethal sectoring frequencies (see Results II).

Recombination and Lethal Sectoring:—The first generation lethal sectors were independent of recombination. This was predictable as the first generation lethal sectors appeared to be due to cytoplasmic damage. Eighteen of the 21 first generation lethal sectors occurred in the daughter cell; this is significantly different than the 1:1 ratio of mother to daughter lethal sectors expected from DNA damage.

Second and third generation lethal sectors were found to be coincident with recombination. This suggests that recombination must greatly increase in some of these cells. The reasoning follows: Recombination of the marked loci occurred in 18% of the pedigrees and shows coincidence with lethal sectoring. If recombination of marked and unmarked loci was randomly dis-
tributed throughout the pedigrees, then the coincidence of lethal sectoring and recombination could not be accounted for. It must be assumed therefore, that some cells, specifically those prone to lethal sectoring, must be showing generalized recombination. Hurst and Fogel (1964) has suggested that two types of yeast cells exist at the time of U.V. irradiation, those which will show single events, and those which are in a "premeiotic" state and will show general recombination. Although the data is too sparse to be statistically valuable, it should be noted that while coincidence of crossing-over on two chromosome arms occurred once, simultaneous crossing-over on three chromosome arms occurred twice, suggesting that multiple events occur much more often than expected (Table IV).

As the cause of lethal sectoring is obscure it is difficult to explain why lethal sectoring and recombination are related. However, if Type II segregations are due to the mechanisms involving unrepaired thymine dimers it is possible that the dimers may eventually result in lethality in some of the pedigree products.
DISCUSSION II

Spontaneous Lethal Sectoring

A variety of strains of haploid *S. cerevisiae* were examined for their colony forming ability. The only strain that showed greatly altered spontaneous lethal sectoring frequencies was the X-ray sensitive and recombination deficient mutant strain, 842-3A (X₁ˢ) of Nakai which showed an increase in spontaneous lethal sectoring by a factor of almost 10. Since statistical analysis is much more sensitive to an increase by factor 10 than a decrease by such a factor, it must also be noted that the X-ray resistant strains of *S. cerevisiae* may have reduced lethal sectoring.

The amount of spontaneous lethal sectoring in the U.V. sensitive strain was not significantly larger than the "wild type" control. This differs from the results of Nasim and Sarenders (1968) who examined two U.V. sensitive mutations of *Schizosaccharomyces pombe* and found that they caused high levels of spontaneous lethal sectoring. It appears then, that U.V. sensitivity and high spontaneous lethal sectoring are not obligatorily associated.

Haefner (1968) has shown that the presence of the U.V. sensitive mutation *uvrA¹³* doubles the spontaneous lethal sectoring frequency of any strain. The presence of the UVS-9 gene may have increased the spontaneous lethal sectoring fre-
quency of strain 227-1b but it is not possible to determine if this was the case as the U.V. sensitive and "wild type" strains had a different genetic background and a small difference in spontaneous lethal sectoring were not meaningful.

Whether the increased spontaneous lethal sectoring present in the strain containing the $X^s_1$ mutant is due to the X-ray sensitivity or the recombination deficiency is not known. Ideally one would want to test an X-ray sensitive strain with normal recombination ability and recombination deficient strain which had no alteration in radiation sensitivity. Mortimer (1968) has isolated a strain which is recombination deficient with normal radiation sensitivity and although pedigree analysis has not yet been attempted, Mortimer (personal communication) has suggested that the strain's growth kinetics suggest a high degree of lethal sectoring.

Since spontaneous lethal sectoring frequencies are increased in the recombination deficient strains, we know that the enzyme systems involved must work in the unirradiated normal cell. This being so, then the recombination system must be considered as a natural "DNA Monitoring System".

**X-ray Induced Lethal Sectoring**

X-rays did not induce lethal sectoring in any of the strains examined. This confirms an earlier report (Haefner 1967b) that lethal sectoring in haploids was not induced by X-rays.
Since dark repair in yeast occurs in all ploidies other than haploids (Patrick, Haynes and Uretz 1964) it can be suggested this induced lethal sectoring in higher ploidies is the result of the dark repair of lethal damage. If this were the case, then a diploid which was deficient in dark repair would be free from induced lethal sectors after X-ray treatment. On the other hand, it is possible that the damage which is caused by X-rays of repair and possibly recombination in the production of lethal sectors has been demonstrated. This confirms recent evidence that some, if not the majority of lethal sectors are the result of nuclear DNA damage (James and Werner 1967, 1969 and Haefner 1968).

The influence of lethal sectoring upon recombination data has been discussed earlier (Discussion I). An illustration of how lethal sectoring influences data from mutational studies is found in the work of Resnick (1968) on the mutation of a "wild type" and a UVS-9-3 strain of haploid yeast. He found that 80% of the "wild type" mutations occurred as whole colony mutants, while only 50% of the UVS-9-3 mutants were of this type. A priori reasoning suggests that lethal sectoring is higher in the "wild type" strain, thus resulting in more "pure" mutant colonies. However, results presented earlier in this study have shown that lethal sectoring is much higher in the UVS-9 mutants.

Considering Resnick's data from this point of view it
becomes clear that the majority of the whole mutant colonies of the U.V. sensitive strain are the result of lethal sectors, while the whole mutant colonies in the "wild type" strain must be due to some other effect.

How mutation affects both products of an irradiated cell is a major problem in molecular biology. It has been proposed that repair of the U.V. mutated strand results in the forming of "pure" mutant clones (Haefner 1967c; Nasim and Auerbach 1967; Nasim 1968). It seems reasonable to conclude then that the "wild type" strain, with its greater repair ability and fewer lethal sectors produces a majority of true "pure" mutant colonies, while the U.V. sensitive strain with reduced repair ability and more lethal sectoring produces simulated "pure" colonies due to lethal sectoring.

This study has shown that lethal sectoring may influence mutation and recombination data. Since lethal sectoring may often be altered in strains with unusual radiation sensitivities, it is important that the effects of these mutants upon lethal sectoring be determined.
SUMMARY I

Pedigree analysis has proven very useful as a technique for studying U.V. induced mitotic recombination. In this study the segregation of markers in 22 recombinant chromosome arms was followed by pedigree analysis; of these, 4 demonstrated a delay between the cross-over and the segregation of the involved markers. To account for this delay, a model involving the exchange of single-stranded DNA was proposed. This model, contrary to the generally favoured models of Whitehouse (1965a) and Holliday (1964), involves the segregation of "long regions" of uncorrected hybrid DNA.

Pedigree analysis follows the fate of cell-lines often revealing lethal sectors. The influence of lethal sectoring upon recombination was found to be considerable when reciprocal processes are involved. In order to remove the bias caused by lethal sectoring, the amount of lethal sectoring that occurs in a strain after a given treatment must be determined.

Recombination and lethal sectoring in the second and third post-irradiation generations, were found to be coincident. This suggests that (1) recombination is greatly increased in certain U.V. treated cells and (2) that the mechanisms of U.V. induced lethal sectoring involves recombination.

From the results obtained in this study as well as data from other sources, it was shown that U.V. treatment results in a recombinant segregation pattern which very rarely, if ever
appear spontaneously.

An hypothesis was made concerning the overall relationships between U.V. and X-ray damage and intergenic and intragenic recombination. The hypothesis proposes that U.V. and X-ray damage are repaired by separate pathways connected by a shunt. The major part of the U.V. damage is corrected by "excision" repair while the greater part of the X-ray damage is corrected by "post-replication" repair. Furthermore, it is proposed that "excision" repair results in reciprocal chromatid exchanges while "post-replication" repair results in single-stranded DNA exchanges which lead to gene conversion and delayed segregation.

The advantage of this model is that it accounts for the different effects which photoreactivation, liquid holding recovery, temperature changes and inhibitors of DNA synthesis, such as fluorodeoxyuridine have upon inter and intragenic recombination. The model also accounts for the effect which a large number of U.V. and X-ray sensitive mutants have upon recombination. Furthermore, this model explains polarity in gene conversion and the basis of X-ray induced fine structure mapping in terms of the repair of heterologous hybrid DNA rather than the formation of hybrid DNA.

Further Experiments:- This hypothesis can be tested by examining a number of predictions based upon the model.

(1) This model suggests that there is a dose effect upon
the type of segregation induced by U.V. irradiation. The greater the U.V. dose the greater the proportion of delayed segregations expected. This can easily be tested.

(2) Photoreactivation is expected to greatly reduce the proportion of delayed segregants. The photoreactivationless mutants of yeast recently isolated (Resnick 1968) present an excellent opportunity to examine this effect.

(3) Since this hypothesis proposes that most X-ray damage is repaired by "post-replication" repair and that "post-replication repair results in delayed segregations, it follows that X-rays should produce primarily delayed segregants. Therefore, X-ray induced mitotic recombination should be examined by pedigree analysis.

(4) This hypothesis predicts that (a) an X-ray sensitive mutant like the X\textsubscript{1} mutant of yeast, which does not show X-ray induced intragenic recombination, may demonstrate U.V. induced intergenic recombination; (b) the X-ray resistant ("wild type") and recombinationless mutant of Mortimer (1968), may not prevent X-ray induced intragenic recombination. Plating experiments would reveal whether this was so.

(5) Mutants which lack "excision" repair and are therefore U.V. sensitive should show greatly increased delayed segregation since "post-replication" repair would need to correct much more damage than usual. Pedigree analysis of such mutants would reveal if this was the case.
Spontaneous lethal sectoring was examined in strains of *S. cerevisiae* which had altered radiation sensitivities. One strain, the $X^S_1$ mutant of Nakai and Matsumoto (1967) showed a significant increase in the amount of spontaneous lethal sectoring compared to the "wild type" strain. An extremely U.V. sensitive strain did not show an altered amount of spontaneous lethal sectoring. Thus, spontaneous lethal sectoring does not appear to be directly related to radiation sensitivity.

However, since the "wild type" strain had much lower amounts of spontaneous lethal sectoring than did the X-ray sensitive strain, it was suggested that the $X^S_1$ mutant was defective in a natural "DNA monitoring system" which operated in the "wild type" strain.

X-rays did not induce lethal sectoring, confirming the results of Haefner (1967b). On the other hand, U.V. did induce lethal sectoring and it was found that at the same survivals, the U.V. sensitive strain showed much greater lethal sectoring than did the "wild type" strain. The suggestion was made that DNA repair was involved in reducing lethal sectoring damage; which indicated a relationship between lethal sectoring and DNA damage.
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