

Mutation and replication in *Ustilago maydis*

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INTRODUCTION

Muller, Carlson & Schalet (1961) have recently pointed out that there is much evidence from *Drosophila* which is incompatible with the recent tendency to believe that a mutagen does not produce a gene mutation directly, but upsets the normal process of replication in such a way that a mutation is formed in a daughter or granddaughter gene as a result of a copy error. Their argument is reinforced by an accompanying paper in which Altenburg & Browning (1961) present new evidence to show that a high proportion of mutations induced by chemicals or X-rays in *Drosophila* are due to stable changes in the treated parental or pre-existing gene. In this paper evidence will be presented for a similar conclusion with regard to mutations induced by ultra-violet (UV) light in the smut fungus *Ustilago maydis*.

In micro-organisms, the ability to screen very large numbers of auxotrophic cells for the presence of rare prototrophic reverse mutations makes it very easy to detect small changes in mutation rate with changing environmental circumstances. This has resulted in the widespread use of the method in studies on the mechanism of mutation in haploid bacteria and fungi. But since only the mutant cell is selected, it is difficult, or impossible, to determine whether both cells of the first division after treatment with a mutagen carry the mutation. Models of mutagenesis which postulate for instance that only one daughter cell becomes mutant cannot be ruled out (e.g. Doudney & Haas, 1959). With suitably marked diploid auxotrophic strains additional information bearing on this point may be obtained. Heterozygous diploid strains of *Ustilago maydis* occasionally yield the reciprocal products of mitotic crossing-over in the first division after UV light irradiation (Holliday, 1961*b*). These somatic segregants are detected as half-and-half mosaic or twin colonies on agar medium. In a diploid which is homozygous for a revertible auxotrophic marker, and heterozygous for other recessive biochemical markers, cells which are induced to mutate to prototrophy by UV light will also occasionally undergo mitotic crossing-over. In such a case there are two possibilities, which are shown in Fig. 1.

If by the use of the appropriate selective media, it is possible to show that a mutant colony consists of the reciprocal products of a mitotic exchange, this indicates that the mutation changed the original gene in such a way that both its daughters were mutant. Whereas if only one of them was mutant, mosaics could not be detected. It should be noted that in order to detect mutant mosaics, the exchange may be in a different arm from *a*, provided it is genetically marked; but that a single exchange proximal to *a* will produce homozygosity for both *a* and *b* loci, and will not therefore allow survival of the reciprocal products of recombination.

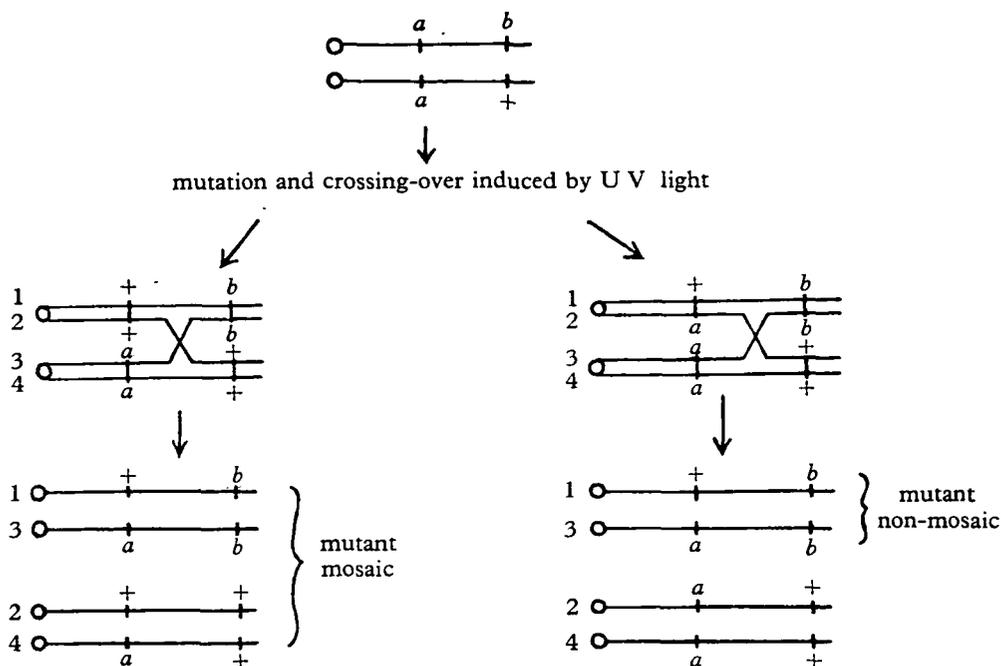


Fig. 1.

METHODS

Reference should be made to earlier papers for the details of methods which are not described here (Holliday, 1961*a*, as modified in 1961*b*).

Strains

Reversions from inositol dependence to independence have been examined in diploids homozygous for *inos-2* and *inos-3*, which are alleles; and from adenine dependence to independence in a diploid homozygous for *ad-1*. These three auxotrophic markers were originally isolated after UV treatment. Other markers used were *nic-3*, *pan-1*, *me-15* and *leu-1*, indicating requirement for nicotinic acid, pantothenic acid, methionine and leucine respectively.

Media

Complete medium has been modified by the addition of 10 mg. inositol per litre. Minimal medium was supplemented as required with the following amounts per litre: inositol, 10 mg.; nicotinic acid, 2 mg., pantothenic acid, 1 mg., adenine, 10 mg.; methionine, 40 mg.; 'Oxoid' hydrolysed casein, 2.5 g. Where *leu-1* was segregating, hydrolysed casein was added and methionine omitted from the selective media; this was because the growth of *leu-1* is inhibited by methionine. The hydrolysed casein did not contain detectable amounts of inositol or adenine.

Mutation induction and assay

Sporidia were spread on plates of complete medium and incubated for 18–24 hours. The vigorously growing cells were then removed from the plates and suspended by vigorous shaking in sterile distilled water. The suspension was concentrated by centrifugation to give a final concentration of about 10^9 spordia/ml. 0.1 ml. aliquots were spread on a series of plates lacking either inositol or adenine, as the case might be, but supplemented with the other relevant growth factors. Plates were irradiated in pairs, 16 cm. from a low pressure mercury lamp providing $1300 \text{ ergs/cm}^2/\text{sec.}$ at this distance. Precautions were taken to avoid photoreactivation. Viable counts were made by washing cells off treated and untreated plates and suspending appropriate dilutions in plates of fully supplemented media. The other plates were incubated for 5–7 days to allow revertant colonies to grow to 2–4 mm. in diameter. Since it was not possible to give an even dose of radiation to more than about 10^8 cells per plate, and since the reverse mutation rates were low in each diploid, usually less than fifty revertant colonies appeared per treated plate. Mutation rates were not increased if the irradiated plates were supplemented with a small proportion of complete medium. Plates were replicated to minimal medium, and the replicas incubated 2 days. Comparison of the replicas with the original plates showed which revertant colonies were wholly segregant for a marker originally heterozygous, and which were mosaic, i.e. consisting partly of auxotrophic and partly of prototrophic cells. Occasional small colony revertants which could not be scored in this way were disregarded. Segregants were identified after removing non-growing cells from the replica plates and growing them on complete medium. Control experiments in which the segregation of non-mutant colonies was followed were carried out as previously described. Any modifications to the above procedures are mentioned in the text.

EXPERIMENTAL RESULTS

(a) *The reverse mutation of inos-3*

A diploid homozygous for *inos-3* and heterozygous for five other biochemical markers was selected from infected maize tissue placed on minimal medium supplemented with inositol (see Holliday, 1961*b*). Its genotype was as follows:

$$\begin{array}{ccccccc} a_1 & pan-1 & & inos-3 & + & & b_1 & me-15 & ad-1 & + \\ \hline & & \circ & & & & & \circ & & \\ a_2 & + & & inos-3 & nic-3 & & b_2 & + & + & leu-1 \end{array}$$

Most of the evidence for the position of the mating type loci, *a* and *b*, and for *nic-3*, *pan-1*, *ad-1* and *leu-1* is given elsewhere (*loc. cit.*). The *inos* locus is linked to *nic-3*, and proximal to it. Similarly *me-15* is linked to *ad-1* and proximal to it. Data showing this, from analysis of the random products of meiosis and from mitotic crossing-over, are given in Table 1. The loose linkage of *me-15* and *leu-1* has been confirmed. The *inos* locus segregates at meiosis independently from *pan-1*; the

evidence for the presence of these loci on the same chromosome is indirect, and depends on the assumption that the haploid chromosome number is only two.

Inos-3 reverts spontaneously to wild-type both in diploid and haploid strains at the very low rate of *ca.* 5×10^{-9} . The rate of reversion is increased nearly one hundredfold after U.V. light treatment, and nearly all the reversions grow vigorously on minimal medium. In a preliminary experiment designed to determine reverse mutation rates in the diploid after various doses of UV light; sporidia which had been grown for 2 days on minimal medium supplemented with inositol, were irradiated insuspension before being embedded in minimal agar supplemented with

Table 1. *The linkage of ad-1 and me-15, and of nic-3 and the inos locus*

(a) Analysis of random sporidia after meiosis:

Cross	Genotypes of progeny				Recombination (%)
	<i>ad me</i>	<i>ad +</i>	<i>+ me</i>	<i>++</i>	
<i>ad-1 me-15/++</i>	110	15	18	73	15.3
	<i>nic inos</i>	<i>nic +</i>	<i>+ inos</i>	<i>++</i>	
<i>nic-3 inos-2/++</i>	60	29	40	65	35.6

(b) Mitotic segregation of various diploids after U.V. irradiation:

Markers in diploid	Phenotypes of segregants		
	Single cross-overs		Double cross-overs
	<i>ad</i>	<i>ad me</i>	<i>me</i>
<i>me-15 ad-1</i>	<i>ad</i>	<i>ad me</i>	<i>me</i>
○ —————	20	25	6
+ +			
<i>inos-3 nic-3</i>	<i>nic</i>	<i>nic inos</i>	<i>inos</i>
○ —————	86	16	0
+ +			

all the growth factors except inositol. Inositol independent reversions which appeared in the plates were picked off, inoculated to complete medium and then replicated to minimal medium. The numbers of reversions obtained were not in fact sufficient to enable accurate estimations of mutation rate to be made. Accuracy was also diminished by the fact that (as sometimes happens when grown on supplemented minimal medium rather than complete medium) the cells were not completely dispersed in the irradiated suspension. The data for various doses have therefore been combined in Experiment 1 (Table 2). Of thirty revertants which were also segregants, fifteen were mixed with cells of wild phenotype. Owing to the method of isolation, it was not possible to determine whether the proportions of auxotrophic and prototrophic mutant cells were equal—as would be expected if they represented the reciprocal products of a mitotic exchange. A surprising

feature was that nearly 10% of the revertants were segregant, a much higher proportion than would be expected for the doses of UV light given.

This experiment showed that the highest absolute yield of mutations was obtained when 50–70% of the sporidia survived, so in nearly all subsequent experi-

Table 2. *Mutation and segregation in the diploid homozygous for inos-3*

		<i>pan-1</i> <i>inos-3</i> +				<i>me-15</i> <i>ad-1</i> +					
		+ <i>inos-3</i> <i>nic-3</i>				+ + <i>leu-1</i>					
		Colonies examined									
Expt. No.	UV dose (min.)	Survival (%)	Mutation rate	Non-mutant		Total segregants		Mosaic segregants			
				Mutant (<i>inos</i> ⁺)	Mutant (<i>inos</i> ⁻)	(No.)	(%)	(No.)	(% of total)		
1	½–3	95–20	2 × 10 ⁻⁸ to 7 × 10 ⁻⁷	341	—	30	8.8	15	50		
2	1	74	1.6 × 10 ⁻⁷	1468	—	25	1.7	10	45		
	2	46	3.2 × 10 ⁻⁷	302	—	5	1.7	4			
3, 4	1	55, 74	—	—	3885	19	0.49	5	26		
	0	100	—	—	2120	0	—	—	—		

Phenotypes of segregant colonies

Expt. No.	Whole colony segregants							
	<i>nic</i>	<i>pan</i>	<i>ad</i>	<i>ad me</i>	<i>leu</i>	<i>me</i>	<i>nic leu</i>	<i>nic pan</i>
1	10	1	—	1	—	1	1	1
2	10	1	1	1	2	1	—	—
3, 4	8	—	—	4	2	—	—	—

Mosaic segregants

Expt. No.	Mosaic segregants					
	<i>nic</i> /+	<i>pan</i> /+	<i>ad me</i> / <i>leu</i>	<i>ad</i> / <i>leu</i>	<i>leu</i> /+	<i>ad me</i> /+
1	15*	—	—	—	—	—
2	11 (6)†	2	—	—	—	1 (1)
3, 4	—	2	1	1	1	—

* It could not be determined whether these segregants were half-and-half mosaics.

† Figures in brackets are the numbers of segregants which were not clear half-and-half mosaics.

ments doses of UV light were used which gave corresponding survival. Although with higher doses than this a higher proportion of mutant segregants might be expected, this advantage would be offset by the fact that the proportion of segregants recovered as mosaics would decrease with dose (Holliday, 1961*b*). In a second experiment (Experiment 2, Table 2) a much larger sample of mutations was

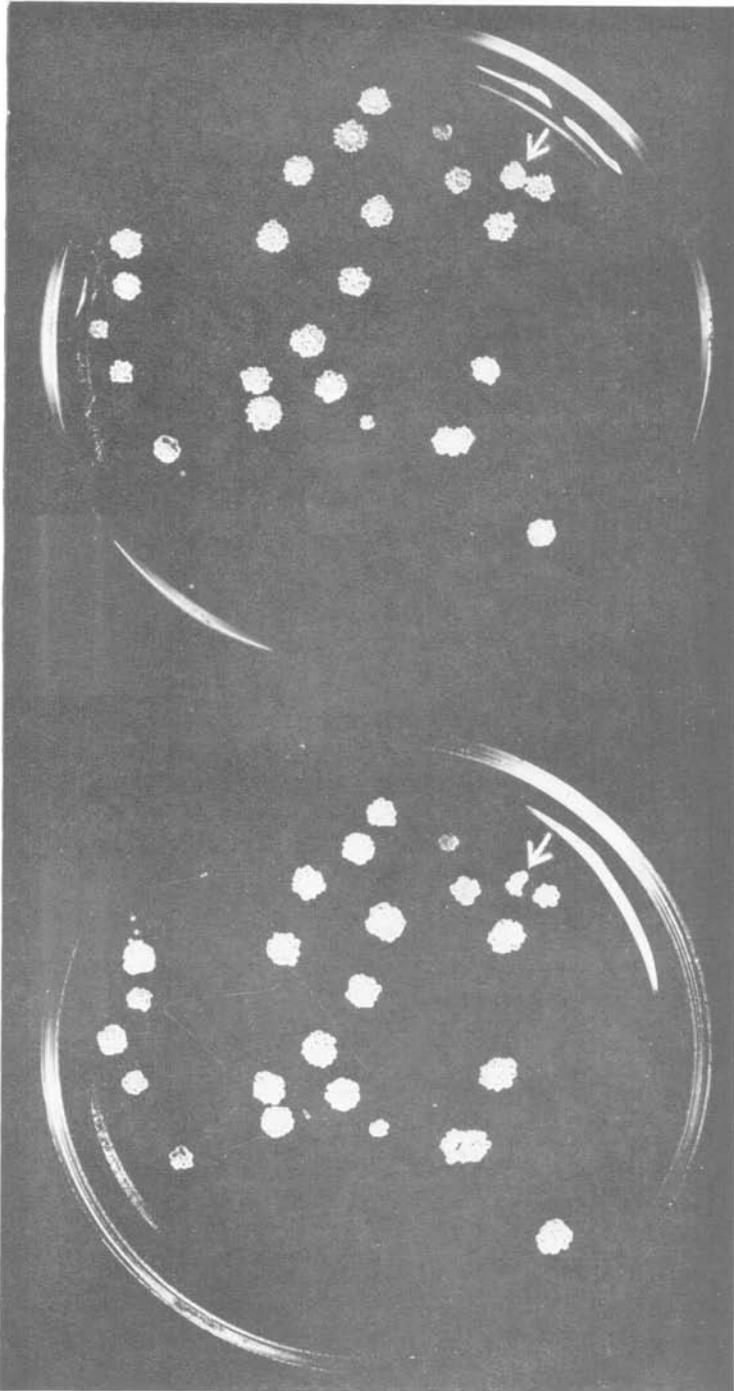


Plate I. Inositol independent colonies derived by induced mutation from a diploid homozygous at the *inos* locus. Mutants were selected on the plate above, which lacks inositol but contains the other relevant growth factors, and replicated to the other plate, which is unsupplemented. One mutant colony is a half-and-half mosaic segregant (see text for full explanation).

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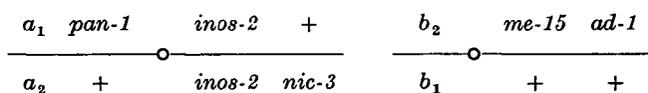
examined, but the proportion of segregants among them was much lower. A number of mosaics were detected, all of which consisted of mixtures of auxotrophic and prototrophic cells. Since these mutant colonies were growing on the surface of agar plates, it was possible to observe by examination of their replicas whether they were half-and-half mosaics. About half of them appeared to be so, and an example is shown in Plate I. The other half were less clear cut, consisting of growing and non-growing cells intermingled in no obvious pattern, and in proportions which were not easy to assess. There was no sharp distinction between the two classes of mosaic. The mixing of auxotrophic and prototrophic cells could be partly or entirely due to the manner of growth of sporidial colonies on supplemented minimal medium. Such colonies tend to have a very convoluted surface, which could result in the overlap of components of a twin colony with a consequent blurring of the division between the two halves on the replica plate.

No mutant colony was detected which consisted of the reciprocal products of a single cross-over proximal to *ad-1*. The only mosaic mutant segregant involving markers on the chromosome not carrying the *inos* locus was presumed to have arisen as a result of a double exchange: one proximal to *me-15*, and one between *ad-1* and *leu-1*. Double exchanges in this arm are quite common (see Table 1).

Although the proportion of segregants among the reversions was considerably lower than in the first experiment, it was significantly higher than in the control experiments (Experiments 3 and 4, Table 2), where non-mutant colonies were examined for segregation. It was surprising that no *nic-3*/+ mosaics were obtained in the controls. This was probably fortuitous, since in every other diploid heterozygous for *nic-3* which has been examined in detail such mosaics have been detected.

(b) *The reverse mutation of inos-2*

A diploid homozygous for *inos-2* was synthesized which had four heterozygous markers in common with the previous diploid:



The spontaneous rate of reversion of *inos-2* was about twice as high as that of *inos-3*, and this was also true of the induced rates of reversion. In three experiments with low doses of radiation a small sample of segregants was obtained amongst reversions. Three of these were clearly half-and-half mosaics, each involving the *pan-1* marker. The data are given in Table 3. Again a control experiment yielded a significantly lower proportion of segregants than that among mutant colonies.

(c) *The reverse mutation of ad-1*

One of the non-mutant segregant colonies from the diploid homozygous for *inos-3* (Table 2) was a twin mosaic, half of which consisted of cells showing segregation for *ad-1*, and the other half for *leu-1*. The evidence is that such colonies arise

Table 3. *Mutation and segregation in the diploid homozygous for inos-2*

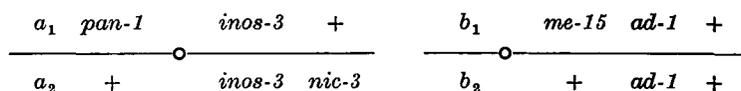
		$\frac{pan-1 \quad inos-2 \quad +}{+ \quad inos-2 \quad nic-3}$				$\frac{me-15 \quad ad-1}{+ \quad +}$			
		Colonies examined							
Expt. No.	UV dose (min.)	Survival (%)	Mutation rate	Non-mutant		Total segregants		Mosaic segregants (No.)	
				Mutant (<i>inos</i> ⁺)	(<i>inos</i> ⁻)	(No.)	(%)		
1, 2, 3	1	71-80	6×10^{-7} *	878	—	11	1.3	2	
2	$\frac{1}{2}$	90	7×10^{-7}	265	—	3	1.1	1	
3	1	71	—	—	1529	3	0.20	0	

Phenotypes of segregant colonies

	Whole colony segregants			Mosaic segregants
	$\frac{nic \quad pan \quad ad \quad me}{+ \quad + \quad + \quad +}$			<i>pan</i> /+
Mutant	7	3	1	3
Non-mutant	2	—	1	—

* Mean value.

as a result of mitotic crossing-over, and therefore the *ad-1* segregant should have lost the *leu-1* marker and have the following genotype:



This genotype was subsequently confirmed. *Ad-1* reverted spontaneously at a higher rate than the *inos* alleles (1.0×10^{-7}), whereas its induced rate of reversion was only slightly higher than that of *inos-2*. A series of experiments were carried out under constant conditions, apart from some variation in the number of cells spread on the plates before irradiation. (With this diploid it was of course necessary to supplement all plates used with inositol.) The combined data are given in Table 4. In the control experiment a low density of cells were irradiated in suspension; this accounts for the lower survival. The segregants among both mutant and non-mutant colonies were of the expected phenotypes. A correlation between mutation and segregation was again observed (2 by 2 $\chi^2 = 6.72$, $P < 0.01$) even though the effective UV dose in the control experiment was higher. The proportion of mosaic mutant segregants was lower than in the previous experiments, and of the ten detected only four were clear-cut twin colonies.

Table 4. Segregation and mutation in the diploid homozygous for *ad-1* and *inos-3*

Expt. No.	UV dose (min.)	Survival (%)	Mutation rate	Colonies examined		Total segregants		Mosaics segregants	
				Mutant (<i>ad</i> ⁺)	Non-mutant (<i>ad</i> ⁻)	(No.)	(%)	(No.)	(% of total)
1-5	1	56-73	8.0×10^{-7} to 1.6×10^{-6}	4850	—	56	1.15	10	17.9
6	1	37	—	—	6152	42	0.68	10	23.8

Phenotypes of segregant colonies

Expt. No.	Whole colony segregants			Mosaic segregants		
	<i>nic</i>	<i>pan</i>	<i>me</i>	<i>nic</i> /+	<i>pan</i> /+	<i>me</i> /+
1-5	29	8	9	7 (5)*	3 (1)	0
6	19	1	12	3	3	4

* Figures in brackets are the number of segregants which were not clear half-and-half mosaics.

Whereas four of the sixteen non-mutant *me-15* segregants were mosaics, none of the nine mutant *me-15* segregants were. This difference may hardly be statistically significant, but it is what would be expected. A single exchange which produced homozygosity for *me-15*, would also do so for the *ad-1* locus, with the result that even if the mutation affected both chromatids, only one daughter cell would survive:

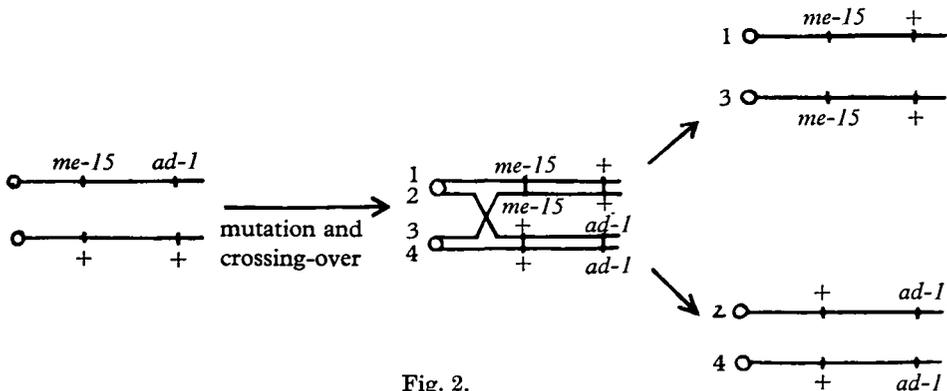


Fig. 2.

If it could be shown that the *ad-1* allele had disappeared from *me-15* segregants, this would provide formal proof for the presence of the mutation in both chromatids. This was investigated by selecting third order segregants from four of the diploids

homozygous for *me-15*, by means of the inositol starvation technique. As in *Neurospora*, cells of *Ustilago* which have a requirement for inositol die very quickly on minimal medium, but if they have an additional biochemical requirement they tend to survive (Lester & Gross, 1959; Holliday, 1962). Sporidia of each diploid were spread on plates of minimal medium supplemented with methionine at a density of about 10^8 per plate. After 2 or 3 days' incubation, inositol starvation was ended by removing the agar with a wide spatula and placing it intact on a plate of complete medium. Rare surviving cells grew to form colonies, which were tested by replication to minimal medium supplemented with methionine and inositol. A high proportion of the colonies were spontaneous segregants with a requirement additional to inositol and methionine. Segregation for *pan-1* is not usually detected by this method since this biochemical requirement does not prevent death by inositol starvation. The results are shown in Table 5. All four strains segregated for *nic-3*, whilst only two did so for *ad-1*. These two, when reverting in the first place from adenine dependence to independence, must either have undergone a mutation which was transmitted to only one daughter chromatid, or less likely, double mitotic crossing-over had left the *ad-1* locus heterozygous.

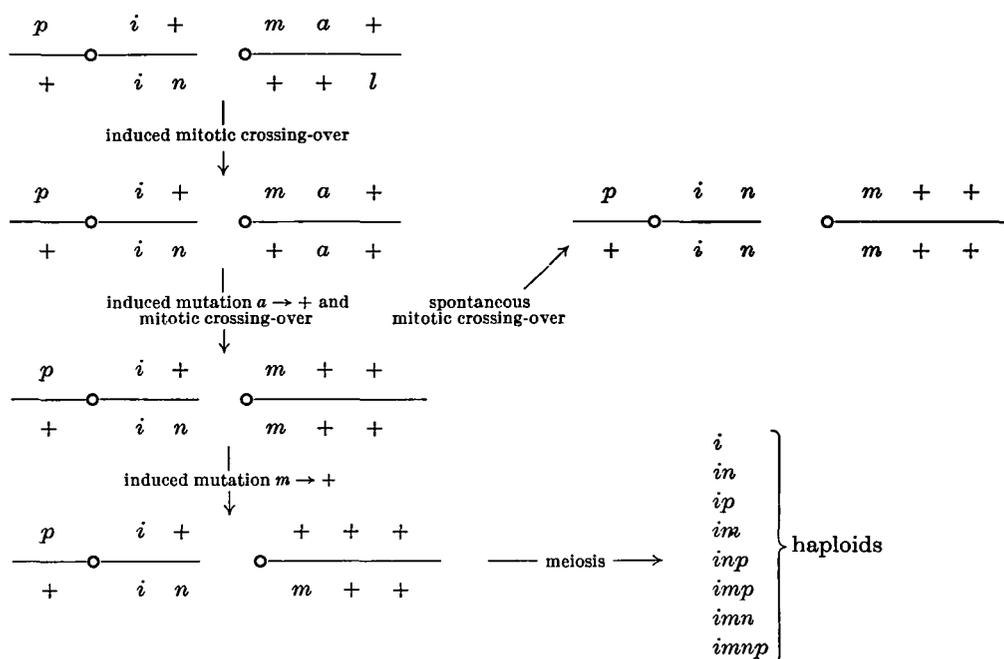
Table 5. Selection by inositol starvation of third-order segregants from ad^+ *me-15* strains (see text)

Strain No.	Segregants examined	Requirement in addition to <i>me</i>		
		<i>ad</i>	<i>nic</i>	<i>nic pan</i>
43.1	57	11	46	0
10.1	34	0	33	1
45.2	86	0	86	0
2.1	25	8	17	0

Although it is easy to show by the starvation method that certain diploids contain the *ad-1* allele, the failure to recover *ad-1* segregants does not completely prove that it is absent. A more certain way of doing this is to put the diploid through meiosis in order to demonstrate failure of segregation of *ad-1*. (Assuming that the mutation to adenine independence was a true reverse mutation and not due to a suppressor.) Such proof was obtained for strain 45.2 by analysing its meiotic products. This could not be done directly, since its requirement for methionine rendered it infertile. However, when a UV induced reversion from methionine dependence to independence was inoculated into maize, it was found to be weakly fertile. A few brandspores were harvested and germinated on complete medium. Sporidia were picked from a number of brandspore colonies and their genotypes identified. Of 159 isolates, all required inositol and none required adenine; whereas segregation for *me-15*, *nic-3* and *pan-1* was as expected.

In recapitulation, the changes through which some of the diploids have gone are

outlined in the following diagram, in which the markers are designated by single letters:



DISCUSSION

With each of the three different reverse mutations which have been examined the same qualitative result has been obtained. Diploid cells in which mutation and mitotic crossing-over have occurred yield some twin mosaic colonies, each component of which carries the mutation. The *simplest* interpretation of these results is that a mutation in a chromosome is transmitted on replication to both chromatids, and therefore that both daughter cells of the first division after UV irradiation are mutant. An alternative explanation is that mitotic crossing-over can be delayed to the second division after irradiation, in which case mutant mosaic colonies would be formed even if only one daughter cell of the first division was mutant. This possibility can be rejected, since there is no evidence that crossing-over can be delayed in this way. If it were, non-mutant diploids which cross-over after irradiation should produce mosaic colonies made up of three components in the proportions $\frac{1}{2}:\frac{1}{4}:\frac{1}{4}$; where the minor components are the reciprocal products of the exchange, and the major component has the phenotype of the parent diploid. If at all common, such colonies would have been detected most easily in experiments with diploids carrying linked markers in repulsion (Experiments 3 and 4, Table 2, and Holliday, 1961*b*).

In earlier work on induced mitotic crossing-over (*loc. cit.*), it was argued on rather indirect evidence that crossing-over might be induced only in cells which had already formed chromatids. This is clearly not true, since the present experiments

show that UV irradiation of a cell in which the chromosomes have not yet divided can stimulate subsequent mitotic crossing-over between chromatids. This suggests that the assumption on which the argument was based, namely that UV light killing is due in large measure to the induction of recessive lethal mutations, was invalid. The correlation between mutation and mitotic crossing-over which has been observed in each diploid, may best be explained by supposing that cells undergo both events at a corresponding stage of the division cycle. The strength of the correlation would be expected to vary depending on the proportion of the division cycle which this stage occupied.

Although there are some data which indicate that the reversion of *ad-1* is not due to a suppressor mutation, direct evidence has not been sought for the other two reversions. Most suppressor mutations are recessive, whereas the mutations to inositol independence are clearly dominant: they therefore most probably represent back mutations at the *inos* locus. However, even if the reversions were due to dominant suppressors, this would not affect the main conclusion; since the formation of mutant mosaic colonies segregant for recessive markers would still occur, provided such markers were not proximal to the suppressor locus.

Since the proportion of mosaics amongst mutant colonies was comparable or higher than that found with non-mutant ones, the simple conclusion is that very nearly all the mutations must be of the type affecting both daughter cells after irradiation. But as has also been noted previously, the segregation data for the same diploid, or for different diploids with common markers, is not always quantitatively reproducible. In addition about half the mosaics did not appear to be clear cut twin colonies, and may possibly have had an origin other than mitotic crossing-over. The only conclusion reached therefore is that at least a substantial proportion of the mutations are not due to copy errors, but to stable changes in the pre-existing gene.

Previous work on this aspect of UV induced mutagenesis in micro-organisms is confusing. The majority of studies have dealt with the kinetics and metabolism of induced mutation in systems in which haploid mutant clones are selected; and where it is not possible therefore to determine whether or not mutations arise as copy errors. Such information can be gained in principle by the use of non-selective systems in which the whole of the surviving population is screened for colonies, or plaques, which are either entirely or partly mutant for a visible character. In practice, in order to obtain a sufficient yield of mutants a high dose of radiation must be used (Witkin, 1951; Newcombe, 1953), or alternatively a highly mutable gene employed (James, 1954; Kaplan, Winkler & Wolf-Ellmauer, 1960). In the former case the irradiation may be expected to interfere with the process of nuclear segregation and thus confuse the result, and in the latter the rates of change are so high as to suggest that a mechanism other than true mutation is operating. The extensive work on the effects of U.V. light on maize (Stadler & Uber, 1942) does not provide a clear-cut answer to this problem either; since most of the genetic changes appeared to be due to chromosome deletions rather than to gene mutation. With a completely different approach, Swann (1962) working with *Schizosaccharomyces*, has

been able to demonstrate that UV irradiation early in the division cycle produces on the whole similar effects on daughter cells, whereas when cells late in the cycle are treated this is not so. The conclusion appears to be similar to that drawn in this paper, namely that UV irradiation often induces stable genetic changes before replication.

During the period in which it was accepted that mutations were due to direct changes in genes, the delayed appearance of a mutation was regarded as an interesting exception (e.g. Auerbach, 1951), now there is a tendency to regard the latter as normal and the former as the exception (e.g. Freese, Bautz-Freese & Bautz, 1961). This change has been entirely due to the recently gained knowledge of the structure and mode of replication of the genetic material (see Drysdale & Peacocke, 1961). If the gene consists of a bipartite deoxyribonucleic acid (DNA) molecule replicating in semi-conservative fashion, it is easy to envisage how induced errors in base pairing could lead to stable mutation only after one or two rounds of replication, and there is now good evidence that this can occur (see Sager & Ryan, 1961). It is much less easy to imagine how both parts of the gene could be affected simultaneously by a mutagen. Muller *et al.* (1961) in dealing with this point, suggest that the breakage of two co-valent bonds followed by the rotation of a base pair, all as a result of mutagenic action, could produce the necessary alteration of the gene. This hypothesis demands a remarkably specific chemical effect by the mutagen: an alternative is suggested in which the specificity of action is accomplished by the cell itself.

There is now good evidence for the induction of distinct chemical changes in DNA by UV light (Beukers, Ijstra & Berends, 1960; Wacker, Dellweg & Weinblum, 1960) and for the removal of these by the photoreactivating enzyme (Wulff & Rupert, 1962). In addition there is evidence from a number of sources for repair mechanisms which act in the dark (Doudney & Haas, 1959; Witkin, 1961; Harm, 1961; Kimball, Gaither & Purdue, 1961), and these may operate in the same way as the photoreactivating enzyme. If U.V. light upsets normal base pairing in DNA, the repair (by definition) must restore it. The hypothesis suggested is that the repair mechanism can sometimes stabilise mutation by *misrepair*, i.e. in restoring normal hydrogen bonding, the base pair substituted for the damaged region may be different from that which was present before the damage occurred. If this was so, then when duplication occurred daughter genes would be identical. If the repair was not effected before duplication, the daughter genes might be dissimilar.

A feature of the hypothesis is that it may help to explain certain anomalies which have recently been exposed. It is difficult to reconcile semi-conservative genetic replication with a copy-choice mechanism of recombination (Delbruck & Stent, 1957; but see Taylor, 1958; Levinthal, 1959). On the other hand, if exchange by breakage and reunion is favoured, then it is difficult to explain gene conversion, or non-Mendelian segregation, in fungal tetrads (see Fincham & Day, in the press). If genetic pairing can occur over short regions at the molecular level, this may involve the separation of the strands of the DNA double helices followed by the annealing of strands from homologous chromosomes. If the annealed region happens to span a

heterozygous site, mispairing of bases will occur. Such a situation may be analogous to that in DNA damaged by UV light. The same repair mechanism may operate, and by adjusting the base sequences in order to restore normal pairing, could bring about gene conversion independent of any recombinational event which may occur in the vicinity.

Another current difficulty in genetic theory has received less attention. Whereas with regard to mutation the gene tends to behave as a single unit; there is considerable evidence both from cytological observation (see Steffensen, 1959; Ris, 1961), and from considerations of the variation in cell DNA content both between genera (Mirsky & Ris, 1952; Sunderland & McLeish, 1961) and even between diploid species of the same genus (McLeish, personal communication), that the chromosomes of higher organisms at least must have a multistranded or polytene structure. (It may not be irrelevant in this context that *Ustilago* has about three to eight times as much DNA per haploid nucleus as several Ascomycete fungi (see Fincham & Day, in the press.)) Thus, assuming that all the DNA is genetic, a mutation must often affect a number of duplicates of the same gene in the same way. This conclusion has often been disregarded on the grounds that it is inexplicable. There appears to be no formal reason why an identical set of parallel and adjacent double helices (gene replicas) cannot exist in at least two different configurations. In the simple case of a pair of helices, strands 1 and 2, and strands 3 and 4 may be hydrogen bonded, or, alternatively, strands 1 and 4 and strands 2 and 3 may be so:



A system such as this may exhibit 'resonance' by alternation between states at certain stages of the cell cycle or life cycle of an organism. If a mutagen damages strand 1, repair may eliminate the damage, but misrepair would result in both strands 1 and 2 being altered. On changing to the other state, the alteration would be transmitted by the same mechanism to the other two strands. Thus a non-specific chemical effect of the mutagen might be converted by the cell itself to a uniform alteration of all copies of genetic material. Provided certain rules govern the ways in which mispairings of bases are corrected, and provided that in a multi-stranded chromosome alternation between states occurs with sufficient frequency, it is at least formally possible to reconcile the cytological and chemical observations with the genetic ones.

SUMMARY

A method is described which makes it possible to detect induced mutations in the pre-existing gene by determining whether both daughter cells of the first division after mutagenic treatment carry the mutation. The method depends on the use of diploid strains of *Ustilago maydis* which have biochemical markers in both homozygous and heterozygous condition. Cells which are induced to mutate

at a homozygous locus will also occasionally undergo mitotic crossing-over. Since only mutant cells are selected, the reciprocal products of such a cross-over will not be detected if the mutation is transmitted to only one daughter cell after treatment. Mutations induced by UV light were examined in three different diploids, and with each, by use of the appropriate selective media and the replica plating technique, it was possible to detect mutant colonies which did consist of the reciprocal products of the exchange. It is deduced that a high proportion of the mutations must occur in the pre-existing gene; and a hypothesis is suggested which attempts to reconcile this conclusion with the current concept of the structure and replication of the gene.

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