STRUCTURE OF THE INCOMPATIBILITY GENE

III. TYPES OF SPONTANEOUS AND INDUCED MUTATION

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CONTENTS

1. INTRODUCTION

PREVIOUS articles in this series have described the quantitative aspects of spontaneous and X-ray induced mutation of the incompatibility gene in *Prunus avium* and *Oenothera organensis* (Lewis, 1948, 1949). The present article deals mainly with the qualitative aspects of mutation in *Oenothera*. It describes the different kinds of mutation which have occurred, spontaneously and under treatment with X-rays, from four different S alleles.

Specific loci used for mutation studies in *Drosophila*, maize or micro-organisms usually have but one wild-type allele. The mutant alleles are recessive and are usually less efficient variants of the wild-type allele. The S locus in self-incompatible plants, on the other hand, has a large multiple allelic series, each allele having its own specific and positive effect. No one member rather than any other of this series can be looked upon as the " wild-type " allele ; rather should the whole series be thus considered. It should be possible, therefore to compare :—

(i) The mutational potentialities of different " wild-type " alleles
(ii) The mutations obtained with the " wild-type " alleles.
(iii) The kinds of mutations produced by X-rays with those arising spontaneously.

Genetical knowledge is still very sparse in regard to many aspects of mutation, and it is a matter of some urgency to fill out this knowledge in view of the present increase in man-made ionizing radiation. It is not only necessary to be able to compare induced mutations with the

spontaneous mutations which are found under present laboratory techniques but, what is more important, to compare both these types of mutations with the gene changes which have been used in the evolution of a balanced genotype ; these we might term *constructive mutations*. They may be the same as the spontaneous mutations we observe in the laboratory or they may be quite different. They may have such small phenotypic effects that our relatively crude methods of detection are useless. Or they may have effects, small or large, expressed only if the particular genetic system concerned is still actively evolving. If this speculation is true the difficulty again lies in our methods, for the genes which our mutation techniques can attack are genes which have big effects, *i.e.* genes nearing the end of their progressive evolution.

The data to be presented are on the changes of a gene which is probably highly evolved. Thus, if our speculation is true, they cannot be expected to reveal the mutational steps by which this gene has differentiated into its present polymorphic state. However, the work may serve to emphasise the necessity for caution when considering the kinds of changes which have been responsible for genic differentiation.

2. METHODS

Most of the methods have been described previously (*loc. cit.*) ; two refinements have been adopted during the present work, genotype testing and seed germination.

(i) *Genotype testing*

The S genotype of a plant was tested previously by self-pollination and reciprocal cross pollination with the six different test plants which are possible with four alleles, *e.g.* S2.3, S2.4, S2.6, S3.4, S3.6, and S4.6. This procedure requires thirteen pollinations for each plant to be tested.

It has now been reduced to four pollinations by using a tetraploid which carries the four different alleles S2.3.4.6. The plants to be tested are selfed, crossed reciprocally with their parent and crossed as pollen parents on to the tetraploid (see table 1).

The six different combinations of compatible and incompatible pollinations are shown in table 1. They are interpreted as follows :—

Results I and II prove that the seedling contains a mutant allele which causes self-compatibility.

The difference between results I and II when the seedling is crossed by parental pollen indicates the relationship of the mutant self-compatibility allele to the other allele in the seedling. For example it has been found that a self-compatible mutant allele retains its action in the style. Such an allele is designated with a prime suffix, thus S_a' is a self-compatibility mutant of S_a. A parent plant, S_{ab}

may have a mutation $S_a \rightarrow S_a'$, the S_a' allele would then pass to the seedling via the pollen. If the other allele of the seedling were S_a then the seedling would be S_{aa}' and this would be compatible with

TABLE 1

Method of genotype testing showing the four pollinations and the five different results

	I	II	III	IV	V	VI
Parental genotype * × Unknown seedling . S_{ab} × S_{ax} or S_{bx} . .	+	+	−	−	+	+
Unknown seedling × Parental genotype . S_{ax} or S_{bx} × S_{ab} . . .	+	−	−	+	+	+
Unknown seedling selfed . . . S_{ax} or S_{bx} selfed	+	+	−	−	−	−
Tetraploid × Unknown seedling . . S2.3.4.6. × S_{ax} or S_{bx} . . .	+	+	−	−	+	−

* The parental genotype may have any pair (S_{ab}) of the four alleles S2.3.4.6. S_x is the unknown allele.

parental pollen S_b. So also will an S_{bb}' seedling be compatible with parental S_a pollen (result I). If on the other hand, the other allele is S_b then the seedling would be $S_a'_b$ and this would be incompatible with parental pollen (result II).

To identify which of the two alleles has mutated the seedlings giving result I or II have to be tested further. Seedlings S_{aa}' or S_{bb}' from result I are crossed by appropriate homozygotes if these are available or by testing whether all or a half of the pollen of test plants S_{ac} and S_{bc} is compatible on the seedling. Similarly if the seedling is S_{ab}' or $S_a'_b$, result II, crossing with S_{ac} and S_{bc} pollen will discriminate.

Results III and IV indicate that the unknown allele is similar to or identical with one of the parental alleles. The seedling is heterozygous in result III and homozygous in result IV. Again two further crosses will discriminate between the homozygotes.

Result V indicates that the unknown allele produces a full incompatibility reaction and is not one of the four alleles used in the experiments. It is a new fully operative S allele.

Result VI proves that the unknown allele is not one of the parental alleles but is one of the other two remaining alleles in the stocks. This may be a true mutation or a contamination by stray pollen from other genotypes. In all mutation work special methods must be used to guard against contamination from unwanted genes. Every precaution is taken in this work to isolate the clones in insect-proof greenhouses. But as a further precaution, only four alleles are kept in the stocks and the nearest plants of this species are 40 miles away. If the unknown seedling gives result VI in the test crosses it is

interpreted as a contamination. Admittedly *bona fide* mutations to the four stock alleles would be overlooked but this is trivial in view of the large number of S alleles in the wild population to which mutations might occur.

(ii) *Seed germination*

Many of the seeds obtained after self-pollination, particularly when there were only one or two seeds in the capsule, germinated with difficulty. The radicle emerged but the cotyledons failed to free themselves from the testa. The routine followed was to dissect off the testa and endosperm before germination and place the naked embryos on sterile pads soaked in Knopp solution in petri dishes. The dishes were placed in a greenhouse and additional light from fluorescent lamps was given for 5 hours each day. After 3-4 weeks the seedlings were ready to prick off into sand, and later into soil.

3. SPONTANEOUS MUTATION

The technique of using incompatibility as a means of detecting mutations of the S gene allows analyses of the mutations at three different levels of viability. At the lowest level there is the number of compatible pollen tubes in self-pollinated styles. This gives a maximum measure of the proportion of pollen grains with a changed incompatibility reaction, including those with an inviable sperm nucleus. The next level is given by the number of seeds produced. This gives an estimate of the number of pollen grains with a mutated S gene and having at the same time a sperm nucleus which is able at least to stimulate the initiation of a zygote. Finally, the number of seedlings produced gives an estimate of the S mutations which are fully viable. Only when the seedlings have been fully tested can we determine which type of mutation has occurred.

It has been shown by the pollen-tube method that there are overall differences between the S mutation rates of $S_4.6$ and $S_3.6$ genotypes (Lewis, 1948). The data in table 2 give the proportion of capsules which contain viable seeds after self-pollination in four genotypes. The fiducial limits for $p = 0{\cdot}025$ calculated by Stevens' (1942) method show that there are significant differences in the number of viable mutations produced by the genotypes. If we can detect which allele of the pair has mutated in each case it is possible to decide whether these differences in overall rates are due to differences in the rates of individual alleles.

In the tests made on the seedlings, if the new allele arising by mutation has some of its original specific properties, then we can attribute the mutation to a particular allele. Fortunately, from this point of view, all the alleles which have come through the mutation sieve can be related to their parental allele either because they still retain their function in the style unimpaired or because they are

apparently identical in all respects with their parental allele. The evidence for assuming the latter to be mutational products will be discussed later after all the data have been presented. Meanwhile they will be referred to as *revertible* mutations.

TABLE 2

Rates of fully viable spontaneous S *mutations obtained from four genotyes*

Diploid genotype	Flowers pollinated	Capsules with viable seeds	Mutations per 10^6 gene divisions *	
				Fiducial limits
S2.6	3,921	1	*0·02*	0·006-0·08
S3.4	931	5	*0·53*	0·17-1·25
S3.6	11,233	9	*0·08*	0·03 -0·15
S4.6	4,717	31	*0·65*	0·51 -0·82

* The number of gene divisions is calculated as $2Nk$, where N is the number of flowers, k is the number of pollen grains per stigma and 2 is a factor which adjusts the figures for the number of gene divisions occurring in the formation of the pollen. In using this factor it is assumed that the number of S genes increases in a geometric series as the power of 2. The last term in this series will be equal to the total of all the preceding terms. It is true that at some early stages in development some of the S genes will be diverted to other tissues and this will have the effect of making the estimate of the number of gene divisions in the descent of the pollen err in the low direction.

Revertibility applies to mutations from a stable allele to an unstable allele which soon reverts at a high rate to the normal. It is quite different from reversibility which applies to mutations from an allele **A** to an allele **a** which is as stable as the original allele but which can mutate back to **A** at a low frequency.

The mutations of all types obtained from four different alleles are given in table 3. It is apparent that, the four alleles show three distinct

TABLE 3

Spontaneous mutations from four alleles

	Flowers	Number of gene divisions	Number of mutations	Rate per 10^6 gene divisions	Fiducial limits
S2	3,921	$19·6 \times 10^6$	0	...	0 -0·05
S3	12,164	$60·8 \times 10^6$	0	...	0· -0·05
S4	5,648	$28·2 \times 10^6$	27	0·9	0·73-1·21
S6	19,871	$99·3 \times 10^6$	19	0·19	0·13-0·25

levels of mutability. The rate obtained with S6 is 0·19 and with S4 it is 0·9 per 10^6 gene divisions.

Assuming that S2 and S3 have a zero mutation rate, calculations

of the expected numbers of mutations to be obtained from the genotypes and numbers of tested alleles given in table 2 are as follows :—

	S2.6	S3.4	S3.6	S4.6
Observed . .	1·0	5·0	9·0	31·0
Expected . .	3·7	4·2	10·5	25·5

The good fit shows that the differences between the genotypes are due to the different mutabilities of the alleles and not to differences in other genes which might modify the mutation rate of the S gene.

Plants derived from the revertible mutations were themselves used in self-pollination to test the mutation rates of the alleles which were derived from one, and in some cases, three generations of selfing.

The data for the derived alleles are given below :—

Allele	Flowers	Gene divisions	Mutations	Mutation rate per 10^6 gene divisions	
					Fiducial limits
S4	339	$2{\cdot}15 \times 10^6$	2	0·9	0·11-3·35
S6	803	$4{\cdot}01 \times 10^6$	2	0·4	0·06-1·80

Clearly these alleles are as stable as their predecessors and there is no sign of a progressive change to a new allele.

In table 4 the revertible mutants have been classified according to the number of seeds in each capsule. This indicates the number

TABLE 4

The number of capsules obtained after self pollination, classified according to the number of seeds they contain : for explanation of the expected ratios see the text

Seeds per capsule	Nuclear divisions	Number of capsules	Expected ratios with constant mutation rate	
1	0	15	23·2	13·12
2	1	3	11·6	6·56
3- 4	2	5	5·8	3·28
5- 8	3	12	2·8	12·24
9-16	4	6	1·4	6·12
17-32	5	3	0·7	3·06
33-64	6	2	0·3	1·53

of nuclear divisions occurring during the time from mutation to the formation of the pollen grains. In other words it is an estimate of the

number of gene divisions occurring between the mutation and the test made to detect it.

It is known that ovaries with less than five fertilised ovules are rarely stimulated to develop, except with the aid of growth-substances. The expected ratios given in columns 4 and 5 of table 4 are based on a constant mutation rate. In column 4 the classes are taken all together irrespective of whether there is any difference between the classes in the viability of the seeds. The fit is not good. In column 5 the classes have been divided into two groups with the 3-4 and 5-8 classes as the dividing line. Here the fit to expectation is much better and is particularly good in the last four groups. These are the groups which should have equal viabilities. The data as a whole therefore fit the expected series and support the revertible mutation hypothesis.

Apart from this the main point of interest is that the revertible mutation can cause a loss of genic activity which will persist for as much as six cell divisions and then at sometime during the growth of the embryo and plant recover its full activity.

There are two exceptions to the general rule that spontaneous mutations are of the revertible type.

(i) A family of plants was obtained from an S2.6 clone by self pollination without treatment. This family contained three seedlings each having a stable S6′ allele. This type of mutation has been frequently found after X-rays.

(ii) A family of five plants was obtained from an S4.6 clone. One plant carried a stable S6′ allele while the others had the reverted S6. This exception is of special interest for the explanation of revertible mutations.

Discussion of the significance of this exception is deferred until after the X-ray results have been described.

4. X-RAY INDUCED MUTATION

Doses of X-radiation ranging from 500-700 *r* units have been given to five different S genotypes. The pollen which subsequently developed from the treated buds was placed on stigmas of the same clone. The frequencies and kinds of mutations obtained are given in table 5. There were two kinds of mutations; the permanent loss mutations and the revertible mutations.

The permanent loss mutation S6→S6′ has occurred six times. All six S6′ alleles are identical in behaviour and differ from the original S6 allele only in their lack of action in the pollen grain. No incompatibility reaction is developed in the pollen grain carrying S6′ but all other characters, such as style reaction, viability, etc., are unaffected. A full description of the tests made on the original S6′ mutation have been described (Lewis, 1949).

A new example of this type of mutation was found in 1950 from an S3.4 plant which had received 700 *r* of X-rays. Two families were obtained, one containing 4 and the other 10 plants. Both families

TABLE 5

Types and numbers of different mutations produced from five genotypes by X-radiation (dose 500-700 r)

Diploid genotypes	Flowers pollinated	Permanent loss mutations		Revertible mutations	
		Type of mutation	Number	Type of mutation	Number
S3.6	870	S6 → S6′	6	S6 → S6	7
		S3 → —	0	S3 → —	0
S2.6	64	S6 → S6′	0	S6 → S6	1
		S3 → —	0	S3 → —	0
S4.6	237	S6 → S6′	0	S6 → S6	1
		S4 → S4′	0	S4 → S4	2
S2.3	117	S2 → —	0	S2 → —	0
		S3 → —	0	S3 → —	0
S3.4	67	S3 → —	0	S3 → —	0
		S4 → S4′	2	S4 → —	0
Total			8		11

had approximately half the plants compatible and half incompatible when used as females with their parents. All the plants were self fertile, and all were compatible as males on to their parent. The plants which were compatible as females were found to be S4.4′ by crossing to a known S4.4 test plant and the plants which were incompatible were S3.4′. Thus the new mutation of S4→S4′ involves only a loss of the pollen activity exactly as in the S6′ mutations and they are classified as permanent loss mutations.

The revertible mutations induced by X-rays are also from S6 and S4. These are similar to the spontaneous revertible mutations described in the previous section.

No mutations of S2 or S3 either permanent or revertible have been obtained with or without X-ray treatment.

If a comparison is made in table 5 of frequency of permanent loss mutations, S6′ and S4′, obtained from different diploid genotypes it will be seen that the allele which accompanies the mutating allele appears to influence the direction of mutation. For example S6 and S4 both give permanent and revertible mutations, but all the stable S6′ and S4′ mutation have come from S3.6 and S3.4 plants, that is, when the mutating allele is accompanied by S3. The genotype S4.6, despite having both potentially mutating alleles has produced no permanent mutations. A calculation of the number of expected S6′ and S4′ mutations from the number of pollinations of S4.6 is as follows :—

$$\frac{6\times237}{870}+\frac{2\times237}{67}=8{\cdot}6$$

which is also the χ^2 for such a deviation with a probability of less than 0·01.

On the other hand the frequency of revertible S6 and S_4 mutations obtained from the S6.4 genotype is not lower than the expected value obtained from the S3.4 and S3.6 genotypes. This is in agreement with the results from spontaneous mutation.

In order that a comparison can be made between the induced and spontaneous mutations, the data are arranged for the 4 alleles in table 6.

TABLE 6

Mutation rates per 10^6 S *genes with* 500-700 *r units of X-rays compared with the spontaneous rate* (R = *revertible*)

Allele	Number of alleles treated *	Mutation type and number	Rate per 10^6 genes	
			Induced	Spontaneous
S6	$1{\cdot}46\times10^6$	S6 (R) 9	5·4	0·19
		S6′ 6	4·1	0·01
S_4	$0{\cdot}38\times10^6$	S_4 (R) 2	2·1	0·9
		S_4' 2	5·2	0·0
S_2	$0{\cdot}08\times10^6$	S_2 (R) 0	0	0
		S_2' 0	0	0
S_3	$1{\cdot}17\times10^6$	S_3 (R) 0	0	0
		S_3' 0	0	0

* The number of treated alleles is difficult to estimate owing to the presence of cells in different stages of development. The estimate made is half the number of alleles tested, this is an overestimate so the induced rates err on the low side.

X-radiation has raised significantly the frequency of both S6′, S_4' and of the revertible S6 mutations.

The frequency of S_4 revertible mutations is higher after X-rays but not significantly so.

One further point to consider with the induced mutations is the relationship between the stage of development of the anther tissue at the time of irradiation and the number of seeds produced in each capsule.

It is necessary to recall that the flowering shoots of *Oenothera* have a graded developmental series of buds, the youngest being at the tip and the oldest at the base of the shoot. The flowers open at daily intervals so that flowers which open soon after irradiation were irradiated at a late stage of development, those opening later were irradiated at an earlier stage. If a mutation has been induced in a late stage of pollen development there will be only one mutated pollen grain and hence one seed in the capsule. The earlier in development the mutation is induced the higher will be the number of mutated pollen grains, hence the larger the number of seeds in the capsule. In fig. 1 the data have been plotted according to the number of seeds per capsule and the number of days from X-ray treatment to flowering.

These data have been collected over a number of years and in consequence the conditions affecting the irradiated plants would be far from constant. The general rate of flower development is dependent on temperature and other conditions, so that it is to be expected that there will be considerable deviation from the expected relationship between time and the stage of development as estimated from the number of seeds produced. This deviation, however, is not enough to obscure the main issues.

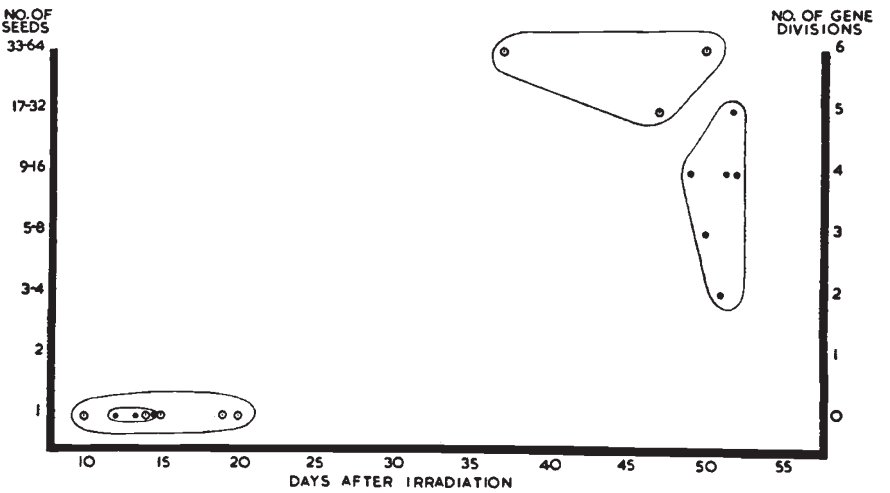

FIG. 1.—The relationship between the number of seeds in the capsules and the time in days from irradiation to pollen maturity. The number of seeds is determined mainly by the number of gene divisions intervening between irradiation and pollen maturity. ● represents *revertible* mutations, ⊙ represents the *permanent* loss mutations.

The single-seeded capsules occur when the interval between irradiation to flowering is 10-20 days. This is true for both permanent loss and revertible mutations.

The capsules with 3-64 seeds occur over a period of 37-52 days after irradiation. Here it is possible to see that the two types of mutations, permanent loss and revertible, occupy different areas of the graph. The significance of this difference will be referred to in the discussion on the nature of the revertible mutations.

The main point to be made here is : that the capsules containing a single seed and those containing many are sharply separated in days after irradiation. This applies both to the permanent loss, S6′ and S_4' mutations and also to the supposed revertible mutations. Thus not only have X-rays produced a significant increase in the revertible mutations over the controls, but these occur in numbers which are in keeping although not identical with the numbers of true loss mutations occurring at different times after irradiation.

5. PSEUDO-FERTILITY OR REVERTIBLE MUTATION ?

The main problem arising out of the present work is the explanation for the functioning in self-pollination of what appear in the next generation to be unchanged alleles. One fact of considerable importance in this connection is the extreme rarity of the phenomenon. For every $2{\cdot}2 \times 10^6$ untreated pollen grains applied to stigmas of *O. organensis* only one or one small group of pollen grains functions. This frequency is commensurate with mutation rates and is lower than would be expected if the cause lay in some environmental effect on incompatibility.

Furthermore, out of the four alleles used only two, S6 and S_4 have shown this temporary inactivation, and treatment with X-rays has also produced mutations only in these alleles. These induced mutations have been of a genuine stable loss type and also of the spontaneous type under discussion. This latter type do not occur at random in time but the numbers are related to the length of time between irradiation and pollen maturity as would be expected if they were true genic changes.

Can these rare S6 and S_4 pollen grains which are compatible on their own style be thought of as due to *pseudo-fertility ?*

Pseudo-fertility, as known in other species, has been attributed, to several causes—end-of-season condition, bud pollination and modifier genes which weaken the incompatibility reaction. The most detailed study on *pseudo-fertility* was made by East (1934) on *Nicotiana Sanderæ.*

He showed that different genotypes had different degrees of pseudo-fertility and that it was increased by pollination in the bud stage. The number of seeds in a capsule after selfing was often as much as one-half and never less than one-tenth the normal compatible complement. The families resulting from pseudo-fertility contained both S homozygotes, showing that both S alleles had functioned on the male side.

In *O. organensis* Emerson (1940) has shown that there is no end-of-season or bud fertility. It is true that allelomorphs S_9 and S_{39} were found to produce incompatible pollen tubes that were longer than normal but no seed was produced. Emerson found also that keeping the plants in the dark weakened the incompatibility reaction slightly but again no seeds were produced. In the course of my own work I have found a slight end-of-season fertility as measured by pollen-tube growth but again there was no effect on seed set.

On the other hand the following summary points to some temporary, and for the time complete, loss of activity of the S allele.

(i) The low frequency of its occurrence.
(ii) The low number of seeds when setting does occur.
(iii) The increase in frequency by X-rays.
(iv) The absence of simultaneous effects on both alleles.

If a loss of **S** activity is the cause then it must persist in some cases for at least six divisions to account for the capsules with more than 33 seeds. The activity must be restored at some time between fertilisation and the formation of the next generation pollen-mother-cells to account for the return to the old allele when tested.

What are the likely causes for the loss of the **S** gene activity?

1. Mutation of a complementary gene or a modifier which when accompanied by a rare combination of other segregating modifiers weakens the action of the **S** gene.

This explanation must be rejected because there would be a great increase in self-fertility in the next generation, and this has not been found.

2. Pollen grains having a vegetative nucleus with a mutated **S** allele and a generative nucleus with a normal allele.

This would explain the occurrence of single seeds but would not explain the presence of groups of seeds in some capsules.

3. A possible cytoplasmic component of the system could temporarily be lost from a cell.

This component would have to be present in meiotic and pre-meiotic cells to explain the loss of **S** activity from a group of pollen grains.

For the present argument it is important to distinguish between two possible cytoplasmic components :—

(i) A non-specific one, that is, one which is a common substrate for all the **S** alleles ; the alleles producing their specific effects in combination with the component after meiosis.

(ii) Components which are specific to each allele. In this case a meiotic or pre-meiotic cell would contain two different kinds of components, one for each **S** allele in the cell.

Whatever type of cytoplasmic component is present it would be distributed to the four cells of the tetrads. If the component is specific to each allele then the irregular segregation which is characteristic of cytoplasmic particles would result in a considerable proportion of tetrad cells receiving only the wrong components. This would lead to a breakdown of the system. Hence, we are left with the possibility of a non-specific component.

A non-specific component would have to be present in numbers sufficient to ensure that all cells of the tetrad always received some. It is unlikely, therefore, that all the components in a cell would be lost by 700 *r* units of X-rays.

The crucial test, however, for such a hypothesis is that the loss of a non-specific component in a pre-meiotic cell would mean the simultaneous loss of activity of both the **S** alleles in the cell.

This would result in mixed families of both homozygotes. These have not been found but unfortunately the numbers tested are not sufficient to decide whether this is due to errors of sampling.

4. The fourth possibility is of a revertible mutation of the S gene.

This explanation is the one that fits the facts better than any other.

The assumptions needed on this basis are (i) that an S allele mutates to an allele which is inactive at least as far as the pollen action is concerned, and (ii) that after some gene reproductions it reverts to its normal activity. The maximum number of gene reproductions to reversion is unknown, but it is known that reversion can occur after as many as six reproductions in the unstable state.

Further evidence for such a reversion is given in fig. 1. In this diagram the relationship between the time after irradiation to pollination and the number of seeds produced is plotted. It will be seen there that for the many-seeded capsules the area covered by the revertible mutations is different from that covered by the permanent loss mutation. The latter, though they arose at a longer interval after irradiation, appear to result from fewer intervening divisions. This could be explained by a reversion of some of the revertible mutants *before* pollination, so that the number of seeds would correspond to less gene divisions than had actually occurred.

One family of seedlings obtained without X-ray treatment is of great significance here. From selfing an S4.6 clone a family composed of plants which were S6.6 and one plant which was self-fertile S6.6′. Thus there were revertible and permanent loss mutations in the same family. It seems that the S gene can mutate to an unstable or segregating condition which can either revert to the old condition or stabilise in the new condition.*

The obvious parallel between this conclusion and the results obtained by Auerbach (1950) from mutations obtained by chemical treatment in *Drosophila* indicates that perhaps the distinction between spontaneous, X-ray and chemical mutations is indeed small.

Auerbach favours the view that chemical treatment may induce a labile premutation which subsequently, after one or more cell divisions, may give rise to the actual mutation or may revert to the old allelemorph.

Darlington (1950) considers the chromosomes to be composed of a number of polypeptide chains, and that delayed mutation is due to the sorting out of mutated from unmutated chains.

The delayed mutations produced by X-rays in *Drosophila* are usually of the half and half mosaic type and these are due to a delay of only one division.

They are readily explained by assuming that two nearby chromosome breaks have occurred and that after the chromosome has split, there has been restitution in one chromatid and a deletion in the other (Muller, 1940).

In bacteria, Demerec and Latarjet (1946) have found that X-rays produce mutations which may not be manifested for as long as 13

* The rare possibility cannot be excluded of a contamination from an extraneous source of S6′ pollen coincidental on the same stigma with the genuine revertible S6 pollen.

divisions. Their explanation lies not in delayed mutation but delayed phonotypic expression.

The only reported cases of delayed mutation after X-rays are, therefore, the one division delays in *Drosophila*. This would appear to weigh heavily against the delayed mutation explanation in *Oenothera*. However, the chromosomal and mutational effects obtained with X-rays are so different quantitatively and to some extent qualitatively in different tissues that this objection is not serious.

6. CONSTRUCTIVE AND LOSS MUTATIONS

Mutation studies, in the past, with their focus on *Drosophila* have been concerned with lethal mutation in a large number of unidentified loci or with changes from a dominant to a recessive allele of a few selected loci. Both these kinds of mutation represent loss changes. Recently attention has been directed to mutations from the recessive back to the dominant " wild-type " allelemorph in *Drosophila*.

These experiments have given entirely negative results (Lefevre, 1950).

With the shift of focus to micro-organisms in the last decade mutations from both wild type to recessive and the reverse have been studied on a much larger scale than has been possible with *Drosophila*.

In *Neurospora crassa* reverse mutations of four genes have been found, but with other genes no reverse mutations could be found or induced (Ryan and Lederberg, 1946 ; Giles and Lederberg, 1948 ; Kölmark and Westergaard, 1949). These reverse mutations are the regaining of the ability to produce a " wild-type " character by an allele which previously had lost this ability. This always leaves the loophole of position effect as an explanation. And in this connection it is significant that the back mutations not only occur spontaneously but are induced by X-radiation and ultra-violet light.

All the mutations, therefore, are either losses of wild type to recessive mutant or a regain of such losses in back mutations. There is nothing new and constructive.

The **S** gene, with its many alleles each having a specific and different effect, would appear to be pregnant with possibilities for constructive mutations. For if the existing alleles in their hundreds have arisen in nature by a mutational process of the kind observed under present experimental conditions then new alleles should arise in experiment.

That none have arisen from 220×10^6 gene divisions which have received no treatment and from $3 \cdot 1 \times 10^6$ which have received 600 *r* units of X-rays throws doubt on the fundamental principles underlying the experiments.

Perhaps constructive mutations detectable with present techniques do not occur.

7. SUMMARY

1. Four alleles of the incompatibility gene in *Oenothera organensis* have been tested for mutation. Two of these alleles have not mutated, either spontaneously or after X-irradiation, in a total of 80×10^6 gene divisions tested. The two other alleles have given two types of mutation : *permanent* and *revertible* mutations.

2. All the permanent mutations involve a loss of the pollen action of the gene only. The stylar action is unimpaired.

3. The revertible mutations enable the pollen grains immediately after mutation to pass the incompatibility test. But these mutations have no effect when tested in the next generation some 15 or 20 divisions later.

4. These temporary changes are probably caused by S mutations to an inactive but unstable form which reverts, either to the normal active allele, or to a stable inactive allele as in the permanent mutation. A cytoplasmic modification cannot, however, be entirely dismissed.

5. Both permanent and revertible mutations occur spontaneously and are induced by X-rays. X-rays induce both types with about equal frequency. Spontaneously the permanent mutations are, however, rarer than the revertible ones.

6. No constructive mutations, *i.e.* to new fully functioning incompatibility alleles have occurred in 223×10^6 gene divisions tested.

7. Tests for small and cumulative changes were made by self pollination for three generations on plants which had arisen from the revertible mutations : no changes in the alleles could be detected.

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