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THE NATURE OF MUTATION IN HEXAPLOID WHEAT*

by

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INTRODUCTION

It was shown by Stadler (1928a, b) more than 40 years ago that mutations are easily induced in diploid plants by ionizing radiation. This finding has since been amply confirmed by the work of many investigators.

Of the mutations which can be induced in diploids, one conspicuous class that occurs in substantial frequency is chlorophyll aberration. Because chlorophyll mutations are easily scored at the seedling stage, they are often used as a measure of mutation rate, and the relative frequencies of different kinds of chlorophyll mutations are used in comparing the effects of different mutagens.

Various lines of evidence suggest that the large majority of the radiation-induced chlorophyll mutations are simply deficiencies for loci essential to chlorophyll formation. There are evidently scores of such loci in each diploid species.

In polyploids of fairly recent origin, such as hexaploid wheat, quite a different situation obtains. Indeed, Stadler (1929) found no chlorophyll mutations at all in hexaploid wheat in an experiment that he calculated would have yielded at least 40 mutations if the material had been diploid. Subsequent experiments of many investigators have confirmed that good, simply inherited chlorophyll mutations are extremely rare in hexaploid wheat following irradiation. MacKey (1954), for example, while obtaining very high frequencies of some so-called peripheral mutations, such as speltoidy, found almost no chlorophyll mutations.

Stadler concluded that the absence of chlorophyll mutations in hexaploid wheat was a consequence of triplication of the loci concerned in chlorophyll production. Deletion of any one locus had no detectable effect because there were still two loci left to carry out that particular step in chlorophyll synthesis. All subsequent results are in accord with this explanation. In particular, it is clear from the normal chlorophyll content of the nullisomics and tetrasomics of the variety Chinese Spring that at least in this variety there is no one locus that is essential to normal chlorophyll production or that leads to chlorophyll abnormality when duplicated.

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In dealing with the nature of mutation in hexaploid wheat, we shall find it useful to classify the genes of wheat according to their degree of duplication and their expressivity at various dosages. As pointed out by Morris and Sears (1967), at least three classes can be distinguished:

(1) Triplicated genes with no increased effect at dosages above four. — (Normal dosage equals six.) As would be expected from the fact that most wild-type genes are dominant in diploids, where a dosage of one equates with a dosage of three in the hexaploid, the large majority of genes in the hexaploid have as great an effect at four doses as at five or six and thus belong in this class. All, or nearly all, of the genes essential to chlorophyll production belong here, along with the other 'vital' genes (MacKey 1954) essential to the viability of the plant. Homozygous deficiency for only one locus has no effect, because four doses remain. These genes are therefore highly intractable to mutation by radiation, but some mutate spontaneously and respond to chemical mutagens.

(2) Triplicated genes with a dosage effect beyond the level of four. — This class is relatively small, but the genes concerned are responsible for most of the characters of the nullisomics. Nullisomy reduces to four the dosage of several different series of these genes, bringing about a particular complex of changed characteristics. Since the other two loci of each series are located, as a rule, on the two homoeologous chromosomes, nullisomy for any one of the three homoeologues has substantially the same effect as nullisomy for either of the other two.

An example of such a series of triplicates is the genes on chromosomes 2A, 2B, and 2D that promote awn development. Each nullisomic has shortened awns and each tetrasomic lengthened awns, but all the possible nullisomic-tetrasomics involving group-2 chromosomes have normal awns. Mutations of such genes are of course easily obtained, a simple deletion or duplication being all that is required.

A few genes in this class may actually show a greater increase in effectiveness at levels beyond six than between four and six. In fact, the awn-promoting genes just mentioned make no awn at all (in the Chinese Spring background) at four doses, produce awns up to 2 mm long at six doses, and make awns up to 20 mm at eight doses. Such genes are more likely to reveal themselves through duplication mutations than deficiency mutations. The *q* series of inhibitors of speltoidy is apparently such a series, at least with respect to its effect on square-headedness. Thus Muramatsu (1963) was able to show that nine doses of *q* had approximately the same effect as four *q* plus two *Q*, and that *Q* was therefore very probably a duplication or triplication of *q*. Deficiencies of *q* in lines with six doses of *q* and none of *Q*, on the other hand, are apparently very hard to detect.

(3) Genes that behave as though they were not triplicated or even duplicated. — Two subclasses may be distinguished here. The first includes loci that have become diploidized through loss or inactivation of one or both of the duplicates on other chromosomes. For example, McIntosh and Baker (1968) studied a line in which nullisomy for chromosome 7B resulted in albinism. This particular line must retain only a single locus (on 7B) of a series essential for chlorophyll production. Similarly, Natarajan et al.

(1958) found a strain of wheat which yielded a high frequency of albino mutations following radiation. Here again the strain concerned has presumably already become deficient for all but one of a series of triplicate loci, perhaps the same series as in McIntosh and Baker's line. Although other examples could be cited, it appears that few series of homoeo-alleles have undergone this kind of diploidization.

The second subclass is more important and consists of genes that have become diploidized through mutation rather than loss. Included here are the few major genes that distinguish the various types of hexaploid wheat: *Q*, which differentiates the aestivum wheats from the speltas; *C*, which distinguishes compactum from aestivum; *s*, which separates sphaerococcum from aestivum; and B_1, B_2, and *Hd*, which suppress awn development. Also belonging here is the gene on chromosome 5B that suppresses homoeologous pairing of chromosomes and thereby makes polyploid wheat diploid-like and stable.

All of the genes in this second subclass are active alleles, whose deficiency has a pronounced effect. They are all believed to be mutants which have occurred since the formation of polyploid wheat. All are easily mutated through simple loss, but such loss mutation only restores approximately the ancestral condition.

Q and B_1 are the only genes in this subclass whose normal alleles have been shown (Muramatsu 1963; Sears 1944, 1954) to have the same effect as the mutant alleles but of reduced intensity. The pairing suppressor is very likely an antimorphic mutation, with an effect opposite to that of the gene from which it arose, and other genes of the group may well be of this type. Although only *Q* and the pairing suppressor are known to have normal alleles which are duplicated on the homoeologous chromosomes, the same may well be true of the other genes of the group.

NEATBY'S VIRESCENT

Of the three classes of genes just described, the first, triplicated genes with little or no increased effect of six doses over four, is of the greatest interest from an evolutionary point of view. Not only is this class by far the largest, but it is the class which is believed to have given rise through mutation to all the major genes acquired during the evolution of the polyploid wheats. Presumably there are other class-1 genes that are capable of mutating in useful ways.

Let us now examine a known mutation of a class-1 gene, Neatby's virescent. This gene is chosen not because of any evolutionary value it might have, but because it has been more thoroughly studied than any other and can perhaps give us some clues as to what to expect at other loci.

Neatby's virescent is a simple Mendelian recessive, v_1, located on the long arm of chromosome 3B (Sears 1956, Steinitz–Sears, 1963). Unlike most chlorophyll mutations in diploids, it is not a mere deficiency for a gene essential to chlorophyll production; it is an active gene with a pronounced dosage effect: one dose (the hemizygote) has no effect under normal conditions, two doses cause virescence, and three doses lead to extreme virescence or albinism.

The normal allele of v_1 proves to be a member of a triplicate series, with V_2 on chromosome 3A and V_3 on 3D (Sears 1957). That V_1, V_2, and V_3 are involved in chlorophyll production was established by combining deficiency for V_1 with nullisomy for chromosome 3A (Sears 1963). The resulting plants, carrying only V_3V_3, were virescent, though less severely so than Neatby's virescent itself. A spontaneous virescent mutation obtained and kindly supplied by Dr. J. G. Hermsen appears also to be deficient for V_1 and V_2 (Sears and Sears 1968), and is, as expected, less extreme than Neatby's virescent.

The mutant allele v_1 competes with its normal allele V_1 and also with V_2 and V_3 (Table 1). Thus $V_1v_1v_1$ is a less extreme virescent than v_1v_1. Tetrasomic 3A or 3D, with two extra doses of V_2 or V_3, is green though v_1v_1. On the other hand, monosomic-3A v_1v_1 is an extreme virescent, and mono-3D v_1v_1 is an embryo lethal. It should be noted that one dose of v_1 cancels the effect of between one and two doses of V. It has the same effect as deficiency for V but is more extreme.

Table 1

Expression of Neatby's virescent v_1 at various dosages of V_1, V_2, and V_3

Genotype	Phenotype
V_1v_1 V_2V_2 V_3V_3	Green
v_1v_1 V_2V_2 V_3V_3	Virescent
$V_1v_1v_1$ V_2V_2 V_3V_3	Less extreme virescent
v_1v_1 $V_2V_2V_2V_2$ V_3V_3	Green
v_1v_1 V_2V_2 $V_3V_3V_3V_3$	Green
v_1v_1 V_2 V_3V_3	More extreme virescent
v_1v_1 V_2V_2 V_3	Embryo lethal

OTHER CHLOROPHYLL MUTATIONS

Several other chlorophyll mutations are now available in hexaploid wheat. First may be mentioned another virescent, induced with ethyl methanesulfonate by Prabhakara Rao and Washington (unpublished) and analyzed by Washington (unpublished). This mutant closely resembles Neatby's virescent but is perhaps a little less extreme. It is located on chromosome 3A, and complementation tests indicate that it is a mutation of V_2 to v_2.

Four *chlorina* mutants have been studied. One of these, *chlorina*-1, induced with EMS by Shama Rao and Sears (1964), is located on the long arm of chromosome 7A (Sears and Sears 1968, and unpublished). Like the virescent mutants, it is an active gene. It causes a slight paleness when hemizygous, yellowness when homozygous, and a gold color when present in three doses. It too competes with its normal allele, for the heterozygote is more nearly normal than the hemizygote.

Driscoll's *chlorina* is a spontaneous mutant allelic to the one just described (Driscoll; personal communication). Its effect is considerably more extreme than that of *chlorina*-1.

Two other EMS-induced *chlorinas* were studied by Washington (unpublished). One proved to be located on chromosome 7B and the other on 7D. Complementation studies indicate that the loci concerned are duplicates of the one on 7A. Thus the *chlorinas* establish a second triplicate series affecting chlorophyll production.

The one other mutant, also EMS-induced, that Washington studied, was also a virescent, but of a different type. It tended to develop white sectors in some of the intermediate leaves. The gene concerned appeared not to be located on any of the group-3 chromosomes.

DISCUSSION

Although more mutants must clearly be studied, it seems fairly safe to say that the genes of hexaploid wheat concerned with chlorophyll production do not mutate at random. As mentioned before, there must be scores of chlorophyll loci in diploid wheat [Smith (1939) induced 23 different chlorophyll mutants in a modest experiment]. We may assume that all or nearly all of these many loci are present in triplicate in the hexaploid, yet all four *chlorina* mutants available involve only a single triplicate series, and the two virescents only one other such series. We may tentatively conclude that most of the chlorophyll genes of the hexaploid mutate at low rates or not at all.

Perhaps we ought to be surprised that any chlorophyll genes in hexaploid wheat can be mutated. The nullisomics show that there are no genes whose absence causes chlorophyll aberration. The mutated gene cannot simply fail to function, as in a deficiency; it must have a more drastic effect. What can happen to a gene that is more drastic than deletion?

Stern (1943) long ago advanced an explanation for the action of genes such as these, which reduce the effectiveness of their normal alleles. From his results with a series of *cubitus interruptus* alleles in *Drosophila*, he suggested that a locus may operate on a limited substrate and that a mutant allele may be very efficient at tying up substrate but very inefficient at converting the substrate to the next product in the sequence. Such an allele is then more effective than a deficiency or a null mutation, because there is such a reduced amount of substrate in the heterozygote that the normal allele cannot function at its full capacity. We may assume that in the extreme case the mutant allele would give rise to no product at all, or to a product which could not be utilized in the process concerned.

In trying to apply the limited-substrate theory to the chlorophyll mutants of hexaploid wheat, we must face the fact that the substrate itself is in all probability under genetic control. Deletion of one of the genes concerned should reduce the amount of substrate and thereby the amount of chlorophyll produced; but we know that there are no genes in hexaploid wheat whose deficiency results in a reduced amount of chlorophyll. It seems best to look elsewhere for an explanation of how a change in a gene can be more effective than a deficiency for that gene.

Now it is important to remember that, while many genes give rise to enzymes, they do not do so directly. The primary product of a gene is messenger RNA, and this, with the aid of a few ribosomes, gives rise to a polypeptide. In almost every case, the polypeptide, called a monomer,

must then combine with one or more other monomers, like or similar to itself, before it becomes an enzyme.

It is reasonable to assume that some at least of the triplicated chlorophyll genes give rise to monomers which are able to combine with each other at random, and thus that the final enzyme molecules are made up of monomers from the different genes in proportions which, for any particular molecule, are determined by chance. It may also be assumed that mutations occur spontaneously (or may be induced by EMS and other chemicals) which result in the production of a defective monomer, and that when such a defective monomer combines with a normal monomer or monomers, a defective enzyme molecule is the result (Sears 1969). Thus the mutant gene itself not only fails to produce normal enzyme, it also prevents a portion of the product of the duplicate loci from doing so. If too little normal enzyme is produced, there will be a reduction in the amount of chlorophyll produced.

With one of three loci homozygous for a defective monomer, the amount of defective enzyme produced will depend upon the number of monomers of which the completed enzyme molecule is composed: the more monomers included in each enzyme molecule, the greater the percentage of the latter with one or more defective monomers (Table 2). Since enzymes are known to be overproduced, sometimes greatly so, it may well be that only genes that give rise to enzymes formed from larger numbers of monomers can be mutated.

There are other circumstances that would render a triplicate series non-mutatable. Obviously the extent to which the enzyme concerned is over-produced would be a determining factor. If only five percent of the enzyme is required for normal function, as has been shown to be true in one instance in *Drosophila* (Glassman and Pinkerton 1960), no effect of rendering one locus defective would be seen even if the enzyme were a heptamer (Table 2).

It is probable that the monomers produced by some triplicate series are sufficiently changed in structure that, while still producing enzymes with the same function, each monomer combines preferentially or exclusively with other monomers from the same locus. Mutation of one locus to a

Table 2

Effect of complexity of enzyme on amount of defective enzyme produced when monomers from triplicate loci combine at random and one locus gives rise defective monomer

No. of monomers per enzyme molecule	Percent defective enzyme
2	55
3	70
4	80
5	87
6	91
7	94
8	96

defective allele can therefore only reduce the amount of enzyme produced by one-third, no more than is accomplished by deletion of the locus.

From the fact that chloroplasts contain DNA, it is reasonable to assume that much of the information required for the synthesis of chlorophyll is coded in chloroplast DNA rather than in the nuclear DNA. Many of the nuclear genes which affect chlorophyll production may then be regulators, which simply turn the chloroplast genes on and off. Since the polynucleotides produced by regulator genes are believed to act directly as monomers, rather than first combining to form polymers, there would be no opportunity for the product of a defective locus to tie up the product of duplicate loci and render them less effective. A defective locus could therefore be no more than equivalent to a deficiency.

It is probable that some enzymes tolerate substantial changes in structure without losing their effectiveness. Mutants would clearly be difficult to get for genes responsible for such enzymes.

Thus the defective-monomer hypothesis predicts that some, perhaps nearly all, of the triplicated genes involved in chlorophyll production in hexaploid wheat should be non-mutatable. With the limited data now available, this prediction appears to be satisfied.

There seems to be no good reason why different mutations at the same locus should not differ in their effectiveness. Monomers defective in different ways may well differ in the ease with which they combine with normal monomers. Also, some types of defect may render an enzyme molecule completely ineffective whereas other defects allow it to retain a portion of its normal function. Thus the fact that Driscoll's *chlorina* is clearly different from its allele *chlorina*-1 is in accord with the defective-monomer hypothesis.

A serious difficulty in the hypothesis lies in the results obtained from EMS treatment of diploid plants. At least some of the many chlorophyll genes of diploids must also operate through the production of multimeric enzymes. Following deletion of one member of a pair of such genes, the remaining normal gene provides enough monomers to form all the enzyme that is needed for normal activity; hence the deficiency mutant is recessive. If, however, one of the genes becomes changed in such a way that it gives rise to a defective monomer, this monomer should combine with normal monomers produced by the remaining normal allele of the heterozygote and give rise to defective enzyme molecules. With random union of defective and non-defective monomers, 75% of a dimeric enzyme would contain one or more defective components, and a level of 97% defective would be reached with only a pentameric enzyme (Table 3).

Therefore some EMS-induced mutants in diploids would be expected to be dominant. But not only have standard mutation experiments with barley and maize, in which an occasional dominant mutant could have been overlooked, failed to yield dominant chlorophyll mutations, but special maize experiments, that could not have failed to reveal any dominant mutants which had been induced, have failed to produce any (Ficsor and Neuffer, personal communication). However, if only a few of the chlorophyll genes actually operate through the production of enzymes rather than regulators, none of these few may involve enzymes which are of just the right degree of complexity to cause a locus producing defective monomers to be effective but not lethal when heterozygous. The virescent mutant v_1,

Table 3

Effect of complexity of enzyme on amount of defective enzyme produced when a diploid is heterozygous for a gene that gives rise to defective monomer

No. of monomers per enzyme molecule	Defective enzyme %
2	75
3	87
4	94
5	97

for example, would probably be a dominant lethal in a diploid, for V_1v_1 should be even more defective than $v_1v_1V_2V_2V_3$, which is already lethal (cf. Table 1).

Whatever the nature of the EMS-induced chlorophyll mutations — whether or not the mutant alleles give rise to defective monomers — it is clear that the chemical produces a kind of mutation in hexaploid wheat not obtainable in significant frequency with radiation. This kind of mutation appears to be equivalent to the simultaneous deletion of two or more members of a series of triplicate loci. By repeated use of radiation over several generations, Kao and Caldecott (1966) obtained chlorophyll defects in hexaploid wheat, but their mutants were presumably not simple in inheritance, and it is likely that there were associated aberrations due to deficiency for more than just the loci of the chlorophyll genes.

What is of course necessary for really progressive evolution is that genes change in such ways as to acquire new functions. Certainly genes have done this in the past, and polyploids have been suggested as organisms in which this kind of change ought to take place relatively frequently (Haldane 1932). This is because gene duplication provides loci which are no longer essential to the organism and are therefore free to mutate to alleles which are radically different, perhaps with a new function. The logic of this reasoning cannot be denied, but we must not assume that changes of the sort indicated occur in substantial frequency, even under the influence of chemical mutagens. Enzymes are extremely complicated, and it must surely be true that constructive changes in them must be very rare indeed, compared to inactivating changes.

Although it may be unrealistic to expect useful frequencies of chemically induced mutations to alleles with new functions, this is not to say that genes of evolutionary value may not be obtained. Since wheat is a cultivated plant, its evolution consists in its becoming more useful to man. This may take the direction of increased yield, wider adaptability, or improved food value. Most of the genes of wheat are apparently triplicated, but there is little reason to believe that this is the best level in every case. Reducing the dosage of some genes may lead to a more favorable balance. And because the chemical mutagens can give rise to mutations that are more drastic than the simple deficiencies caused by radiation, the chemicals may well prove to be the more useful mutagens.

SUMMARY

Several simply inherited virescent and *chlorina* mutations have appeared in hexaploid wheat, either spontaneously or following treatment by ethyl methanesulfonate. The virescent mutations involve triplicate loci on homoeologous chromosomes 3A, 3B, and 3D, and the *chlorinas* triplicate loci on 7A, 7B, and 7D. The mutant genes are not simple deficiencies but are active alleles, each of which competes with its normal allele and with the normal allele's duplicates on the other two homoeologues. Each mutant gene is thought to give rise to a defective polypeptide (monomer), which may combine with normal monomers from the normal allele and its duplicates to produce a defective multimeric enzyme molecule. In this way a mutant gene can be as effective, or more so, than simultaneous deficiency for two of the three loci concerned.

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Note added in proof. – The unpublished works referred to in the section 'Other Chlorophyll Mutations' is now in print, as follows:

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