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Suppression of 'purple' in *Coprinus lagopus*—an anomalous genetic situation

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1. INTRODUCTION

Although inter-allelic complementation has been studied at many well-defined loci specifying particular enzymes (see review by Fincham, in press), there is no extensive data on inter-allelic complementation between mutants having more subtle effects upon the phenotype, such as modifiers and suppressors. Nor is it clear what results are to be expected from complementation tests between mutants in which a gene has not suffered a permanent structural alteration but which has had its expression modified by virtue of its position in the genome, or its proximity to a controlling element. The work reported here is a study of the functional and structural relationships between fifty spontaneous suppressors of the mutant purple in the basidiomycete *Coprinus lagopus* and is relevant to the structural and functional organization of the genome as a whole.

2. MATERIALS AND METHODS

The wild-type stocks of *C. lagopus* used were originally collected in Hertfordshire by Professor D. Lewis. They are H1 (mating type A_5B_5), H2 (A_6B_5), H5 (A_5B_6) and H9 (A_6B_6) and were isolated as single basidiospore cultures from the same fruit, thus being closely related but not isogenic. The life-cycle is well described by Lewis (1961).

All plate cultures and slants were incubated at 37°C. Fruiting was carried out on sterilized dung, the dung bottles being incubated for 2 days at 37°C. and then at 26°C. The complete medium used throughout was a modified Fries medium, as described by Day (1959).

Three purple mutants (*pur*) have been used which were derived independently from wild-type. Mutants *pur*-1 and *pur*-2 were both obtained from A_6B_6 wild-type with ultra-violet light as mutagen. Dr P. R. Day kindly supplied *pur*-3, obtained from the same wild-type culture by treatment with β-propiolactone. All of these cultures had, when isolated, the same characteristic appearance. They were slow-growing colonies with an irregular scalloped edge, little or no aerial growth, few oidia

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and a colour deepening from pale purple in the young colony to almost black in the old one. There is a marked tendency for the mycelium to grow in the form of rhizoids giving the colony a straggly appearance to the naked eye. The hyphae branch more frequently than wild-type. The pigment diffuses freely into the agar and its absorption spectrum and behaviour in simple chemical tests are compatible with it being a quinone. A rigorous characterization of the pigment has not been attempted. Various progeny purples, from crosses of the original mutants with A_5B_5 wild-type, are much fluffier than the originals and in some cases the onset of pigmentation is delayed until the colony is quite well grown.

3. RESULTS

(i) *Genetic characterization of purples*

All three *pur* mutants give white dicaryons with wild-type, indicating that they are recessive. The dicaryons (*pur*-1 × *pur*-2), (*pur*-1 × *pur*-3) and (*pur*-2 × *pur*-3) are all purple, indicating functional allelism of the three mutants. All purple dicaryons have proved infertile, behaving in this respect like dicaryons homozygous for most auxotrophic mutants. The white dicaryons formed between purples and wild-types fruit normally, giving 1:1 segregation for purple and white. Scores (purple/white) where 166/172 for *pur*-1, 63/67 for *pur*-2 and 120/134 for *pur*-3. Scoring of mating type in the progeny of these crosses showed that *pur*-1 and *pur*-2 segregate independently of both the mating-type loci. *Pur*-3 however appears to be closely linked to the *A* locus. Of seventy-one progeny tested thirty-five were recombinant for *pur*-3 and *B* but only one was recombinant for *pur*-3 and *A*. That one of three functionally allelic mutants should show linkage with a marker while the other two do not is an anomaly relevant to the results to be discussed later. The most obvious explanation is that the *pur*-3 mutant, which differs from *pur*-1 and *pur*-2 in having been produced by a radio-mimetic chemical mutagen, carries a chromosomal aberration in addition to (or possibly constituting) the purple mutation.

Two dicaryons of the type *pur* × wild were also resolved into their component monocaryons by isolating the monocaryotic hyphae produced by germinating chlamydospores (Lewis, 1961). These were then scored for colour and mating type. Colour segregated exclusively with the parental nuclear genotypes (Table 1). The

Table 1. *Monocaryons recovered from dicaryon chlamydospores of constitution* pur × *wild*

Dicaryon	Resolvants
A_6B_6 *pur*-1 × A_5B_5 wild-type	38 purples, all A_6B_6
	39 whites, all A_5B_5
A_6B_5 *pur*-3 × A_5B_6 wild-type	15 purples, all A_6B_5
	39 whites, all A_5B_6

evidence, of regular segregation in basidiospores, recovery of parental monocaryons from dicaryons and linkage of *pur*-3 to *A*, clearly rules out the possibility that the purple phenotype is due to cytoplasmic mutation

(ii) *Instability of purple cultures*

The most interesting characteristic of purple cultures is their instability which is expressed as the appearance of white sectors during the growth of the colonies. When sectors appear in young colonies, they are usually faster growing than the purple from which they arise. However, sectors appearing later in the development of the colony, when the surrounding medium is already well pigmented, do not display their full growth rate until sub-cultured onto fresh medium. The idea that this is due to inhibition of the white sector by some product of the purple colony is supported by the observation that purple colonies do inhibit the growth of wild-type colonies growing alongside.

When the white cultures derived from sectors are mated with a compatible purple stock, the dicaryons produced are purple. When they are mated with wild-type, normal white dicaryons are produced, many of which, when fruited, segregate some purples among the progeny. Clearly therefore the sectoring is due to recessive suppressor mutations rather than to back mutation. That suppression also is not due to any sort of cytoplasmic effect is shown by the regularity with which resolution of (purple × suppressed purple) dicaryons yields the purple and suppressed components correctly associated with their original mating-type alleles (Table 2). Although

Table 2. *Monocaryons recovered from chlamydospores of purple dicaryons of constitution* pur +/pur su

	Monocaryons	
Dicaryons	Purple	White
1. Purple A_5B_6 × suppressed A_6B_5	2, both A_5B_6	16, all A_6B_5
2. Purple A_6B_6 × suppressed A_5B_5	2, both BA_{66}	7, all A_5B_5

pur-1 and *pur*-3 monocaryons are recovered with reduced efficiency as compared with suppressed monocaryons, all twenty-seven monocaryons are of the expected mating type.

There is very considerable variation between individual suppressed cultures with respect to vigour and appearance. This applies not only to sectors arising from different purple stocks but also to distinct sectors arising in the same purple colony and to suppressed cultures segregating from crosses to wild-type which do not necessarily resemble the parental suppressed culture. This variation is clearly not due solely either to variation in the background genomes involved or to specific differences between individual suppressors.

(iii) *Complementation between suppressors*

The functional relationships between fifty independent suppressors of purple were studied using complementation in dicaryons. The result of mating two suppressed purples is either to give a purple dicaryon (i.e. complementation between suppressors allowing expression of the homozygous purple condition) or to give a

white dicaryon (i.e. no complementation between suppressors). After about 5 days there is a clear distinction between those dicaryons which have acquired a deep reddish purple colour and those which have not.

Fifty white sectors were isolated from purple basidiospore colonies, one sector only

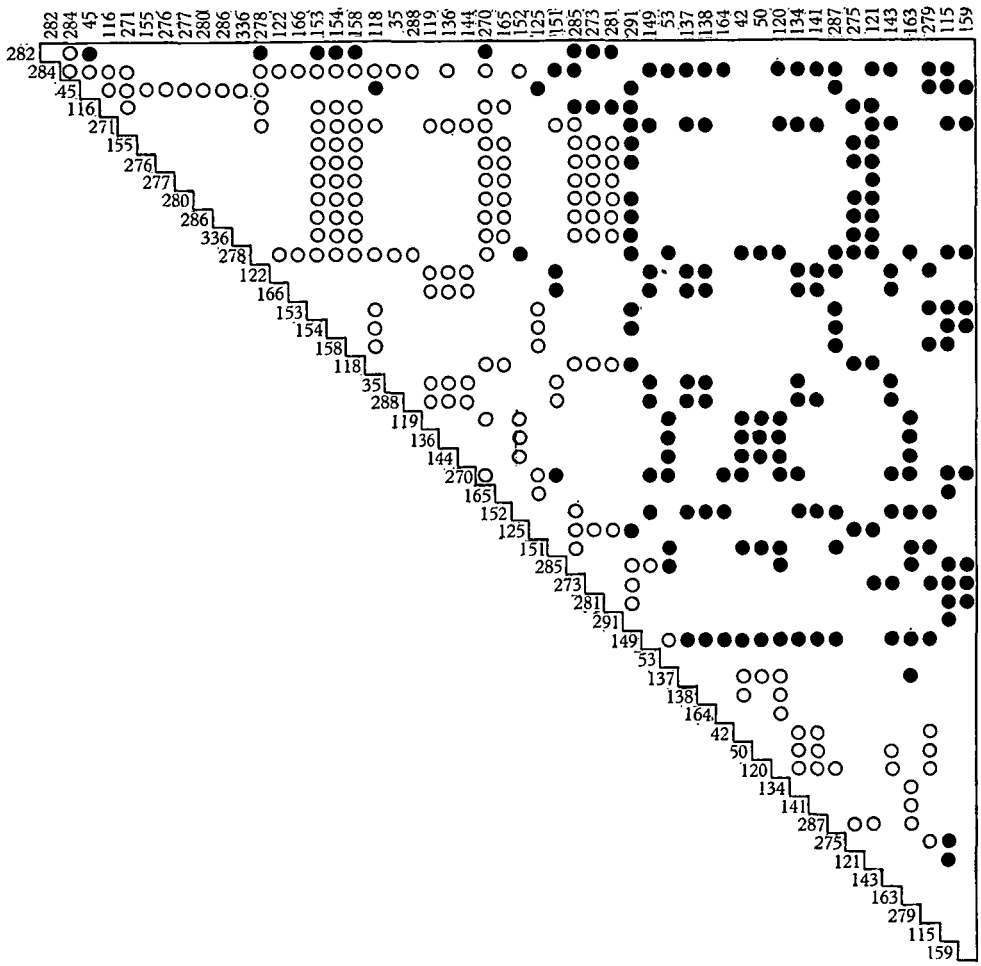

Fig. 1. Complementation matrix involving fifty suppressors of purple. ●, complementation; ○, no complementation.

being taken from each purple. The basidiospore cultures carried *pur*-1, *pur*-2 or *pur*-3 (thirty, eight and twelve cultures respectively) and included all four mating types A_5B_5, A_5B_6, A_6B_5 and A_6B_6. Two suppressors (Nos. 50 and 53) were exceptional in that they were isolated from sister sub-cultures of the same purple and are thus isogenic. All fifty cultures were tested, as described above, for the presence of *pur* together with a recessive suppressor.

The complementation tests were made by simply mating appropriate pairs of cultures together on complete medium and scoring the colour of the resulting dicaryons. Tests were routinely carried out in duplicate and colonies of the com-

ponent monocaryons were grown separately under the same conditions, to provide colour controls. It should be emphasized that there is no question in this system of failure to complement being due to a failure of heterocaryon formation. Dicaryon formation was recognized by the presence of clamp connexions in all cases. Possible tests with the original sectors were restricted by mating type. Thus all the A_5B_5 cultures could be mated with all the A_6B_6 cultures and all the A_5B_6's with the A_6B_5's giving two independent matrices of results. These two sets of data were linked together by the use of mating-type recombinants of the original suppressed cultures,

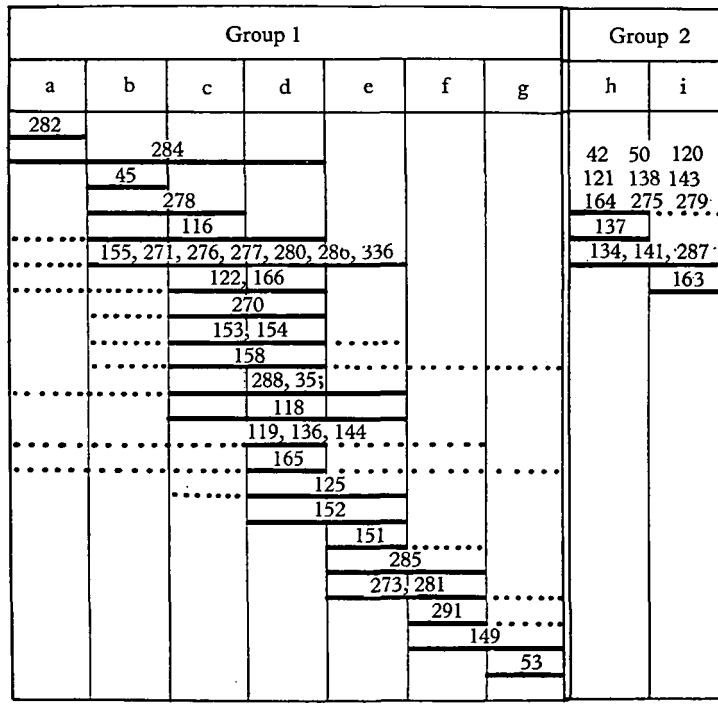

Fig. 2. Complementation map corresponding to data of Fig. 1.

although a complete analysis, requiring all cultures to be available in each of two mating types, was not attempted. The results of the complementation tests are presented as a matrix (Fig. 1) and as a conventional complementation map (Fig. 2). Uncertainties in the latter, due to the incomplete nature of the data, are represented by dotted extensions to the bars of the map. Extension of the solid bars along the dotted lines is possible, but not necessary to satisfy the existing data.

It will be seen that thirty-four of the fifty cultures are related to form a continuously overlapping series (Group 1), i.e. fall upon one complementation map having seven complementation units. Of the remaining sixteen cultures, fourteen (comprising Group 2) complement all Group 1 cultures with which they have been tested but are themselves split into two complementation units by the single positive reaction of 137 with 163. The remaining two cultures (115 and 159) have given a positive reaction in all tests done but 115 could be placed into unit *i* of Group 2 and

159 into either unit of Group 2 or into unit *a* of Group 1. Thus no additional units are required to accommodate these two cultures. They are not, however, included in Fig. 2.

There are at least twelve distinct patterns of complementation reactions among Group 1 cultures and at least three among the Group 2 cultures. Further data could conceivably introduce more complexity into the map but could not make it more simple. It may also be pointed out that the degree of interlocking between thirty-four members of the map is such that no great simplification is to be gained by rejecting, as spurious, one or two results. If only those thirty suppressed cultures carrying *pur*-1 are considered, the map is simpler but essentially similar to that of Fig. 2. Allele specificity on the part of the suppressors does not appear to be an important factor.

The most obvious conclusion to be drawn from these data is that the thiry-four suppressors of Group 1 and the fourteen of Group 2 form two series of functional alleles, each showing inter-allelic complementation.

If the suppressors of each group do constitute allelic series, then clearly they should all map the same locus on the genetic map. In fact the complementation results were obtained first and it was then, surprisingly, found that although all the suppressors appeared to be linked to *pur*, those of Group 1 do not apparently map at a single locus.

(v) *'Linkage' between suppressors and purple*

No fruits have yet been obtained from crosses homozygous for *pur*, either in the presence or absence of suppressors. The infertility of purple dicaryons was not so surprising but the infertility of the vigorous, apparently wild-type, dicaryons homozygous for *pur* and carrying two non-complementing suppressors was unexpected.

Crosses of suppressed purples to wild-type thus comprise the only source of information on the linkage of the purple locus and its suppressors. (It may be possible at a later date to get indirect information with the use of other linked markers.)

Progeny of crosses of forty-one suppressed purples (and some of their derivatives) with wild-type were screened for the segregation of purple colonies by random basidiospore platings onto complete medium. A low colony density was used to avoid any scoring error due to overgrowth of one colony by another and plates were examined daily for 7 days to ensure inclusion in the score of both early and late pigmenters. Errors due to early suppression of *pur* segregants, or early dicaroytization with a neighbouring colony, were unavoidable but unlikely to have been appreciable in practice. Of the colonies on the plates 50% carried *pur* and there would be thus a 50% chance that any potentially purple colony dicaryotizing when young would give a purple dicaryon and a lesser, variable, chance that a spontaneously suppressed purple would also dicaryotize to give a purple dicaryon on old plates. The appearance of a purple dicaryon on a plate where a purple monocaryon had not already been recorded prove to be a very rare event.

The results of these crosses are expressed graphically in Fig. 3. The immediately striking features of the results are:

(1) the apparent linkage of all suppressors to the gene suppressed (independence being expected to give 25% purples);
(2) the division of the suppressors into two major groups—twenty three (Group A) giving purple segregations of from 2% to nearly 20% and eighteen Group B) that are very close to *pur* as judged by the rarity of purple segregants (e.g. 1/1623 for 287; 3/1694 for 159; 3/421 for 279);
(3) the way in which the percentages in Group A vary continuously from 2% to 20%.

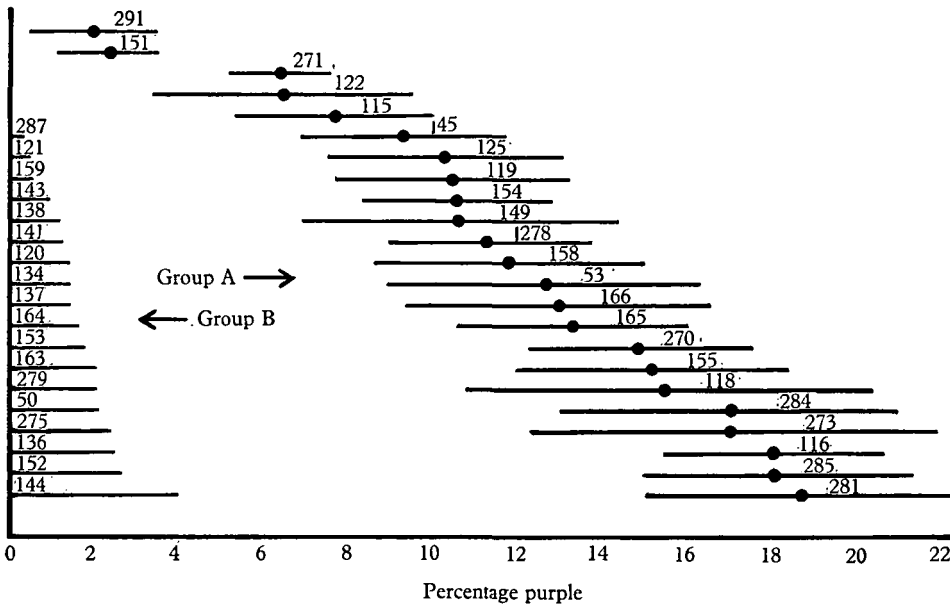

Fig. 3. Segregation of purples from crosses of forty-one suppressed cultures with wild-type. Results given either as points with bars extending to 95% confidence limits (Group A), or, as bars extending from zero to upper limit of true probability at 5% level of significance (Group B.)

Of the eighteen suppressors in segregation Group B, fourteen are also distinguished by forming complementation Group 2. It therefore seems that there are two distinct varieties of suppressors, differentiated by both procedures. The four remaining Group B suppressors, which fall into complementation Group 1, could represent the low end of the continuous distribution of values of Group A. Within Group A, there is no correlation between the position on the complementation map of any suppressor and its characteristic purple segregation.

Basidiospore viabilities varied considerably between different crosses but there is no correlation between these viabilities and the corresponding purple scores. A few of the crosses had viabilities approaching 100% and in those cases in which different crosses involving the same suppressor gave markedly different viabilities, purple

scores were very homogeneous. The observed variation in purple segregations is not, therefore, due to differential viability effects. Neither is it likely to be due to variation in background genome, especially in view of the large difference between those cultures derived from a common purple stock (i.e. fifty and fifty-three) and the consistent values obtained with mating-type recombinants of several suppressed cultures (Fig. 4).

When the results are broken down according to the *pur* allele involved, it is clear that the distribution of values is very similar in the case of *pur*-1 and *pur*-2 but that

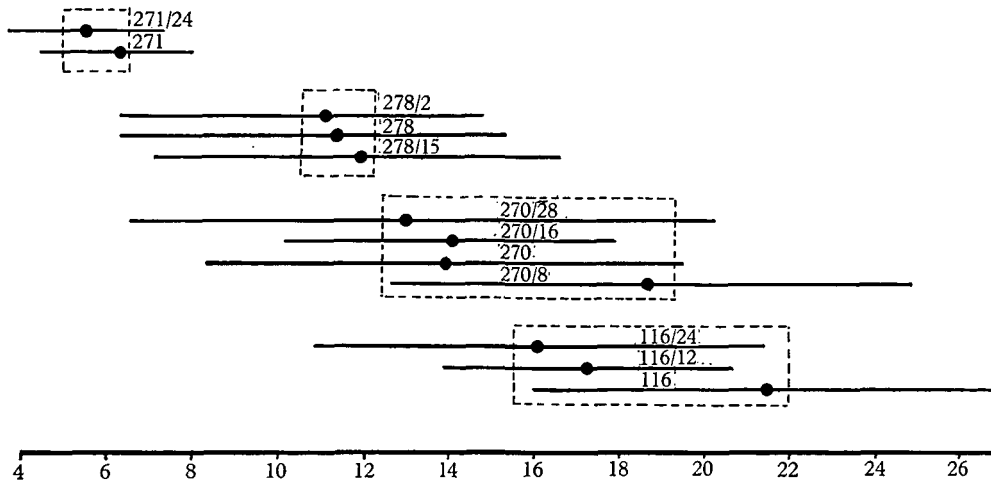

Fig. 4. Illustrating the degree of correspondence, with respect to segregation of purples, between each of four suppressed cultures and mating-type recombinants derived from them.

with *pur*-3 there is a marked increase in the fraction of values in the 0–3% range, the fraction being 7/22 for *pur*-1, 2/7 for *pur*-2 and 9/12 for *pur*-3. (9/12 and 7/22 not homogenous in 2×2 table, $\text{chi}^2 = 4{\cdot}05$, $P = 0{\cdot}05$.) The linkage of *pur*-3 to the *A* mating-type locus would not of necessity imply a disturbance of the purple suppressor region in the *pur*-3 stocks, but in view of this difference between them and the *pur*-1 and *pur*-2 stocks it is probable that there has been some disturbance.

The data formally indicate linkage between all the suppressors and the gene suppressed. If the most economical assumption is made, that all the suppressors lie on the same side of the purple locus, and if normal reciprocal recombination obtains, then the purple suppressor region has a map length approaching forty map units. However, it remains to be demonstrated unequivocally

(1) that suppressors are single gene mutations,

(2) that the segregation of purples from crosses of suppressed purples to wild is due to normal reciprocal recombination,

(3) that any two suppressors, both linked to *pur* as judged by the data of Fig. 3, are themselves linked.

Crosses between cultures homozygous for purple and heterozygous for suppressors have invariably proved infertile but direct estimation of linkage between suppressors in the presence of homozygous *pur*$^+$ ought to be possible. Resolution of these uncertainties will depend upon the ability to identify and use cultures of genotype *pur*$^+$ *su*$^-$ and will be facilitated by tetrad analysis. Work to this end has been started.

4. DISCUSSION

The data may be summarized as follows. Fourteen of the fifty suppressed cultures studied do not differ greatly with respect to the number of purples segregated in crosses with wild and fall into one functional group, Group 2, with only one case of what may be considered to be inter-allelic complementation. Of the remaining thirty-six cultures, two cannot be allocated on the data available and thirty-four constitute the series of 'functional alleles' of Group 1. The twenty-six of these tested differ widely with respect to their purple segregations. If the percentage purples segregated be taken to indicate a variable degree of linkage between the Group 1 suppressors and the *pur* locus, then the results are anomalous in that a series of functionally allelic mutants are scattered over a considerable length of the linkage map. In particular, fifty-two pairwise combinations of Group 1 suppressors do not complement but the members of each pair give significantly different purple segregations. Of these, fourteen pairs are homozygous for *pur*-1.

Two other examples have been reported of non-complementing genes being positionally distant. One involved a pair of suppressors of *met*-1 in *Coprinus lagopus* (Lewis, 1961) and the other a pair of loci in *Drosophila* affecting sternopleural chaeta number (Gibson & Thoday, 1962).

One kind of explanation for this would suppose that the problem is a quantitative one. For example, there could be several suppressors affecting the metabolic pathway concerned, their effects being additive. For suppression to be effective, one dose of *su* would be required for each dose of *pur*, this 1:1 relationship being maintained in the double heterozygote. However, since some suppressors do complement and others do not it would be necessary to assume some quantitative variation between individual *su* mutant alleles and it at once becomes very hard to explain all the data. Consider, for example, the behaviour of cultures 45 and 285 as set out below.

	291 (2%)	284 (17%)	271 (6%)	282 (?)
45 (9%)	+	−	−	+
285 (18%)	−	+	−	+

(+ = complementation, − = no complementation)

When tested with 291, 45 is + and 285 is −, a result which could be expressed as 45 > 285, implying that 45 produces more of some metabolite and 285 less. (This could, alternatively, be expressed in terms of incomplete recessivity of one or other

of the mutants.) When tested with 284 however, the opposite result is obtained, 285 > 45. When tested with 271 and 282 the two cultures are equivalent. Although an explanation for the data may eventually have to be sought in these terms, it seems hardly profitable to discuss it further in the absence of any information at the metabolic level and in the absence of any data on the effect of two suppressors in *cis* as compared with *trans*.

Another type of explanation is that the suppressor mutations all fall into a large unit of structure and function, analogous to the bacterial operon (Jacob & Monod, 1961). There is a formal similarity between the situation in a bacterial heterozygote, where an 0° mutation in *trans* to a mutation of one of the structural genes of the operon gives a mutant cell, and the situation of two non-complementing suppressors which are distant from each other. The operon in bacteria is, however, still a very small unit, a few genes long, and it would be stretching the concept very hard to extend it to a region apparently forty map units long in a fungus.

Even if an explanation in terms of several discrete suppressor loci could be squared with the complementation data, the series of Group A segregation values (Fig. 3) would remain suspiciously continuous in nature. There are two possible approaches to the explanation of this continuity.

(i) *One suppressor locus*

(*a*) It could be argued that these values represent a series of more or less distorted estimates of a true *pur*/*su* interval and that different mutational events at one point in the genome (the *su* locus) could have a differential effect upon the frequency of recombination in this interval. If one disregards the unlikely possibilities, first, that the *su* locus could be specifically concerned with recombination mechanism as well as pigmentation, and second, that a series of structural aberrations within one locus could have such marked differential effects upon recombination, then such events would have to be aberrations with one break point only affecting the *su* locus. But in this case the gene product would be an incomplete polypeptide and the complementation data hard to explain, since the mechanism of complementation involves differently altered rather than incomplete polypeptide chains.

(*b*) The different purple segregations could alternatively be due to different rates of mutation of a gene concerned with pigment production. (That such events should be scored as segregants at meiosis, but not as purple sectors during vegetative growth of suppressed cultures, is not surprising in view of the slow growth of purple cultures on plates. However, one might expect to observe a similar rate of mutation in single oidial isolates from suppressed cultures, and this has not been found.) Genes affected by variegated position effect (Lewis, 1950) or controlled by the transposable elements of maize (McClintock, 1956) frequently exhibit enhanced mutability, as do genes subject to 'paramutation' (Brink, 1960). If the mechanism underlying the segregation of purples from crosses of suppressed purples to wild is mutation, rather than recombination, then the different allelic suppressor mutants could *either* themselves have high and specific back mutation frequencies *or* have specific mutagenic activities, acting upon some other gene to restore pigmentation.

(ii) *Suppression caused by changes at any point in a large chromosomal region*

An alternative approach is to suppose that a genetic change *at any point* in a large chromosomal region can affect pigment production. There are only two systems in which this kind of situation has been demonstrated.

In the classic cases of variegated position effect (Lewis, 1950), genes displaced from their normal position in the genome display an instability of expression in the somatic tissues. This effect, which is reversible, is associated with proximity of the gene(s) concerned to specific heterochromatic regions of the chromosome and the effect may spread along the chromosome for a considerable distance to affect an array of different genes. It is thus conceivable that an aberration having a break point anywhere within, for example, forty map units of *pur* could alter pigmentation by virtue of a position effect and a series of such changes could display the observed range of purple segregation values. The nature of 'position effect' will, eventually, have to be explained in terms of the mechanisms of gene action and relationships between different position effects which simulate inter-allelic complementation may yet be recognized.

There are also the complex situations described in maize and, more recently, in *Salmonella*, where the expression of a gene may depend upon its proximity to certain genetic elements which are transposable from place to place in the genome (McClintock, 1956; Dawson & Smith-Keary, 1963). If suppression were due to the insertion (or removal) of such a 'controlling element' at varying positions within forty map units of *pur*, then the complementation and segregation data might be reconciled, with 'inter-allelic' complementation occurring between different "alleles' of the element. (Or, between the different expressions of a gene resulting from activity of the *same* element in different positions—a possibility analogous to that discussed above with respect to position effect.) There is no evidence for such transpositions in this material and tests of these several hypotheses will be dependent upon the use of well-marked chromosomes which are not yet available. The heritability of the characteristic purple segregations in suppressed cultures does not discount the possibility of transpositions, since the outgrowth of white sectors is a selective process whereas only a very limited sample of recombinants of any one suppressed culture have been tested for segregation of purples.

SUMMARY

The spontaneous reversion to white of three purple mutants of *Coprinus lagopus* has been demonstrated to be due to suppression. Forty-one of the fifty suppressed purples isolated were crossed to wild-type and all crosses gave fewer than 25% purple progeny, i.e. all the suppressors appeared to be linked to the gene suppressed. Values for the percentage of purples segregated from these crosses varied continuously from 0% to nearly 20%. The functional relationships of the fifty suppressors were investigated by the technique of complementation in dicaryons. The pattern of results formally indicate two suppressor loci with extensive inter-allelic

complementation at one of them. The functional allelism indicated by the complementation results is not compatible, in conventional terms, with the apparent positional spread of the suppressors. Several possible explanations are discussed but more data are required before a choice can be made between them. It seems most probable that the same genetic entity is involved in each independent event of suppression and that the characteristic segregations of purples from crosses of suppressed purples with wild-type will have to be explained in terms other than of normal recombination.

The author wishes to thank Professor D. Lewis for introducing him to fungal genetics and for many helpful discussions with respect to this problem. A detailed account of this work comprised the author's Ph.D. Thesis (London, 1964).

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