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*OBSERVATIONS ON THE BEHAVIOR OF SUPPRESSORS IN NEUROSPORA**

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Communicated by G. W. Beadle, January 14, 1952

A suppressor of pyrimidineless *3a* (37301) and some aspects of the behavior of the suppressed mutant have been described earlier.[1] The observation that lysine, ornithine, citrulline and arginine influence growth responses of the suppressed mutant suggested studies of the behavior of recombinants involving *pyr 3a* and *s* and mutants having requirements for these amino acids. Effects of the pyrimidineless mutant and its suppressor upon certain lysine-requiring mutants have been reported.[2] The present paper deals with a somewhat greater variety of interactions observed between *pyr 3a* and *s* and mutants which utilize proline, ornithine, citrulline or arginine.[3] These interactions include suppression of two non-allelic prolineless mutants by the pyrimidineless suppressor and partial suppression of *pyr 3a* by three non-allelic ornithineless mutants.

Effects of the pyrimidineless suppressor on mutants of the proline-arginine series.—The suppressed pyrimidineless mutant, *pyr 3a-s* (37301-s) was first described[1] as being characterized on minimal medium by a lower growth rate than that of wild type, and by stimulation by pyrimidine, lysine or histidine. It has since been found that this is true only of some isolates. Others grow as rapidly as wild type, still others very nearly so, and the stimulations are very slight. The reported inhibition by ornithine, citrulline and arginine and its release by lysine remain properties of the recent isolates, however, although there may be differences in the effectiveness of the compounds as inhibitors. Proline is also inhibitory but very slightly so.

Crosses of *pyr 3a-s* were made to the following strains: 21863, prolineless; 35401, 44207 and 51506, which use proline, ornithine, citrulline or arginine;

27947, 29997 and 34105, which utilize ornithine, citrulline or arginine; 30300 and 33442, which utilize citrulline or arginine. For convenience these mutants have been renamed in the following fashion:

prolineless

21863 = *prol 1* 35401 = *prol 2* 44207 = *prol 3* 51506 = *prol 4*

ornithineless

27947 = *orn 1* 29997 = *orn 2* 34105 = *orn 3*

citrullineless

30300 = *cit 1* 33442 = *cit 2*

Growth responses and genetic relationships of these strains have been described by Srb and Horowitz,[3] Srb[4] and Srb, Fincham and Bonner,[5] who have shown that, except for *prol 3* and *4*, which behave as alleles, strains among these which use the same compounds are genetically distinct.

Ascus dissections were done on minimal agar plates as described by Haas *et al.*,[6] and the spores were heat treated and allowed to germinate on the plates. The germinated spores were transferred to tubes containing minimal agar medium supplemented with cytidine and proline or arginine and the resulting cultures classified by means of tests in tubes of liquid minimal medium with appropriate supplements. Members of spore pairs were isolated and cultured together as reported by Fincham[7] and Haskins and Mitchell.[8]

In tables 1 and 2 the types of asci observed are tabulated so as to show the phenotypes, but not the order in the ascus, of the spore pairs. The ornithineless and citrullineless mutants are designated as *arg* in order to simplify the tabulation in table 2. The suppressor, *s*, is represented only in those spore pairs in which its presence could be detected phenotypically by the qualitative classification tests used. From table 1 it may be seen that from the crosses *pyr 3a-s* × *prol 2*, *3* and *4* the prolineless mutant appeared in only one of the four spore pairs in asci of types 2, 5, 7 and 12, and in types 3, 6 and 10 it did not appear in any spore pair. Hence, it seems that *prol 2*, *3* and *4* are suppressed, and the types of segregation observed are consistent with the view that they are suppressed by *s*. This was confirmed by recovering the three prolineless mutants from out-crosses of phenotypically *pyr-s* strains derived from asci of types 2 and 3, and also by recovering them from out-crosses of phenotypically wild strains from asci of types 10 and 12. For the sake of clarity it should be pointed out that only one of the *pyr-s* spore pairs from a type 2 ascus, and only one + spore pair from a type 12 ascus carried the prolineless mutant, since it appeared, unsuppressed, in one spore pair in asci of these types.

There is no indication that *prol 1* is suppressed since it appeared in two spore pairs from each ascus, although in types 4, 9 and 11 it presumably

combined with *s* in at least one spore pair, as evidenced by the appearance of unsuppressed *pyr*. As table 2 shows, the same result was obtained with the ornithineless and citrullineless mutants since they appear to have combined with *s* in asci of types 2, 4, 5 and 6. The presence of *s* in an unsuppressed *arg* strain from each cross was demonstrated by crossing these to *pyr 3a* and recovering *pyr 3a-s*.

TABLE 1

CROSSES OF *pyr 3a-s* TO *prol 1, 2, 3* AND *4*

	TYPES OF SEGREGATION				*prol 1*	*prol 2*	*prol 3*	*prol 4*
1	*pyr-s*	*pyr-s*	*prol*	*prol*	1	3	..	5
2	*pyr-s*	*pyr-s*	*prol*	+	..	2	1	4
3	*pyr-s*	*pyr-s*	+	+	..	1	1	4
4	*pyr-s*	*pyr*	*prol*	*prol*	1	..	..	..
5	*pyr-s*	*pyr*	*prol*	+	..	3	2	..
6	*pyr-s*	*pyr*	+	+	..	3	..	1
7	*pyr-s*	*pyr-prol*	+	+	..	..	4	..
8	*pyr-s*	*pyr-prol*	*prol*	+	2	1	3	..
9	*pyr*	*pyr*	*prol*	*prol*	1	..	..	..
10	*pyr*	*pyr*	+	+	..	2	..	2
11	*pyr*	*pyr-prol*	*prol*	+	2	..	..	..
12	*pyr*	*pyr-prol*	+	+	..	3	5	5
13	*pyr-prol*	*pyr-prol*	+	+	1	6	1	2
					8	24	17	23

NOTE: Only the phenotypes of spore pairs are shown, not the complete genotypes nor the order in the ascus.

TABLE 2

CROSSES OF *pyr 3a-s* TO *orn 1, 2* AND *3* AND *cit 1* AND *2*

	TYPES OF SEGREGATION				*orn 1*	*orn 2*	*orn 3*	*cit 1*	*cit*
1	*pyr-s*	*pyr-s*	*arg*	*arg*	7	..	..	4	5
2	*pyr-s*	*pyr*	*arg*	*arg*	1	..	1	1	2
3	*pyr-s*	*pyr-arg*	*arg*	+	1	3	1	5	..
4	*pyr*	*pyr*	*arg*	*arg*	..	..	..	2	3
5	*pyr*	...	*arg*	*arg*	..	..	..	2	1
6	*pyr*	*pyr-arg*	*arg*	+	..	5	2	..	..
7	*pyr-arg*	*pyr-arg*	+	+	4	2	2	2	..
					13	10	6	16	11

NOTE: Only the phenotypes of spore pairs are given, not the complete genotypes nor the order in the ascus.

Growth responses of recombinants from the crosses were further tested by measuring dry weights of mycelium from cultures in 125-ml. flasks containing 20 ml. of Fries medium[9] with appropriate supplements. The suppressed prolineless mutants *prol 2-s*, *prol 3-s* and *prol 4-s* are not distinguishable from wild type on minimal medium and are not affected by

added proline or arginine. The suppressed double mutants *prol 2-pyr 3a-s* and *prol 3-pyr 3a-s* were not found to differ from *pyr 3a-s*.

Tests of recombinants of *s* with the ornithineless and citrullineless mutants have shown that *s* affects these in a way which may be considered the opposite of suppression. Three of these mutants, *orn 3*, *cit 1* and *cit 2*, grow slowly on unsupplemented medium, and in about 10 days reach a dry weight which approaches that of wild type after a 4-day growth period. As would be expected, they have lower requirements than do *orn 1* and *2*, which do not grow on unsupplemented medium. All of the five combinations with *s*, however, fail to grow a weighable amount on unsupplemented

TABLE 3

REPRESENTATIVE DRY WEIGHTS (IN MG.) FROM 125-ML. FLASK CULTURES AT 25°C. AFTER 4 OR 10 DAYS' GROWTH WITH THE FOLLOWING SUPPLEMENTS IN 20 ML. OF MEDIUM: ORNITHINE = DL ORNITHINE HCl, 1 MG.; CITRULLINE = DL CITRULLINE, 1 MG.; ARGININE = L ARGININE HCl, 1 MG.; CASEIN = ENZYME-HYDROLIZED, 40 MG.

	0		ORNITHINE		CITRULLINE		ARGININE		CASEIN
DAYS.....	4	10	4	10	4	10	4	10	4
orn 1	0	0	12	..	21.5	..	30.5	..	57
orn 2	0	0	9	..	27	..	29.5	..	..
orn 3	2	65	53	..	56	..	51	..	61
cit 1	3	40	2.5	50	63	..	46	..	58
cit 2	5.5	66	4	61	57	..	42.5	..	68.5
orn 1-s	0	0	0	tr.	13.5	..	21	..	39
orn 2-s	0	0	tr.	tr.	14	..	28	..	..
orn 3-s	0	tr.	tr.	tr.	20	..	33	..	40.5
cit 1-s	0	0	0	0	15.5	..	23	..	42
cit 2-s	0	tr.	0	tr.	19.5	..	31	..	47
pyr 3a	0	0	0	0	0	0	0	0	0
pyr 3a-orn 1	0	0	tr.	13.5	1.5	3.5	tr.	23.5	6
pyr 3a-orn 2	0	0	tr.	9.5	tr.	2	tr.	18	..
pyr 3a-orn 3	2.5	105.5	tr.	49.5	4.5	39	tr.	44.5	26.5
pyr 3a-cit 1	0	0	0	0	0	0	0	0	0

NOTE: tr. (trace) represents a quantity of mycelium which is visible but too small to be removed and weighed.

medium in 10 days, and give about the same response to citrulline or arginine as *orn 1* and *2*. Furthermore, the recombinants *orn 1-s*, *orn 2-s* and *orn 3-s* all fail to respond to ornithine even at a level which is 3 to 4 times higher than that required for half-maximum growth of *orn 1* and *2*. Hence the presence of *s* appears to convert these five mutants into strains with absolute requirements for citrulline or arginine. This is shown by data given in tables 3 and 4.

The effect of ornithineless mutants on the pyrimidine requirements of pyr 3a.—In classification tests of the progeny from the crosses considered in the preceding section the behavior of the double mutants of *pyr 3a* and *orn 1*, *2* and *3* suggested that they lacked the absolute pyrimidine require-

ment which is characteristic of *pyr 3a*. These double mutants were therefore obtained from crosses of the three ornithineless mutants to *pyr 3a* in order to make it certain that *s* was not present. Results from tests of these recombinants in flasks appear in tables 3 and 4. In *pyr 3a-orn 3* the pyrimidine requirement appears to be completely suppressed on unsupplemented medium, since this strain grows in the same manner as does *orn 3*. On medium supplemented with ornithine, citrulline or arginine growth is somewhat slower than on minimal medium, but more rapid growth was obtained with enzyme-hydrolyzed casein as supplement. *Pyr 3a-orn 1* and *pyr 3a-orn 2*, like *orn 1* and *2*, fail to grow on unsupplemented medium but grow slowly without pyrimidine when supplied ornithine or arginine, until they reach the dry weight characteristic of the

TABLE 4

REPRESENTATIVE DRY WEIGHTS (IN MG.) FROM 125-ML. FLASK CULTURES WITH 20 ML. OF MEDIUM, AFTER 4 DAYS' GROWTH WITH THE SUPPLEMENTS GIVEN BELOW

	0	1 MG.	2 MG.	4 MG.	8 MG.
	DL Ornithine Hydrochloride				
orn 1	0	13	24	44	80.5
orn 2	0	11	22	35.5	44.5
orn 3	2	57	54	59	65.5
orn 1-s	0	0	0	0	0
orn 2-s	0	tr.	tr.	tr.	tr.
orn 3-s	tr.	tr.	tr.	tr.	tr.
	Cytidine Sulfate—0.5 Mg. DL Citrulline				
pyr 3a-orn 1	0	3.5	6.5	18	73
pyr 3a-orn 2	0	4	49.5	77	65
pyr 3a-orn 3	3	64.4	89	82.5	63
pyr 3a-cit 1	0	2	2.5	1.5	1,5
pyr 3a	30.5	32	33	31	32

NOTE: tr. (trace) represents a quantity of mycelium which is visible but too small to be removed and weighed.

single ornithineless mutant on that level of the supplement. They grow even more slowly with citrulline as supplement, as would be expected for reasons which are given in the next section. After 14 days, however, with 4 mg. of DL citrulline per flask, the following dry weights were obtained: *pyr 3a-orn 1*, 109 mg.; *pyr 3a-orn 2*, 115 mg.

The difference in the rate of growth of *pyr 3a-orn 3* on minimal medium and on medium supplemented with ornithine, citrulline or arginine suggests that these compounds interfere with suppression of *pyr 3a* by *orn 3* as they do with suppression of *pyr 3a* by *s*. This is also suggested by the observation that growth of *pyr 3a-orn 3* is more rapid with lower than with higher levels of ornithine. The effect of lysine on the *pyr 3a-orn* double mutants has not been thoroughly investigated, but it appears to be com-

plicated by inhibition of the *orn* mutants by lysine. A significant stimulation of *pyr 3a-orn 3* by 6 mg. of L lysine hydrochloride was observed when this strain was cultured in the presence of 2 mg. of DL ornithine hydrochloride. With this ratio of lysine to ornithine *pyr 3a-orn 1*, *pyr 3a-orn 2*, *orn 1* and *orn 2* were completely inhibited whereas growth of *orn 3* was less affected. Thus it seems that lysine may exert the same effect on the *pyr 3a-orn* double mutants as it does on *pyr 3a-s* but that this is obscured by its inhibitory effect on the *orn* mutants.

It is considered unlikely that a change in the *pyr 3a* gene, such as back-mutation, is responsible for the apparent suppression of the pyrimidine requirement in these double mutants. The absence of an absolute requirement for pyrimidine was detected in *pyr-orn* double mutants among the progeny of six crosses involving two different isolates of both *pyr 3a* and *pyr 3a-s*. Segregants carrying *pyr 3a* alone, on the other hand, even those derived from asci which also produced double mutants, showed the absolute requirement. As a further check, the isolates of the double mutants which were used in the above experiments were crossed to wild type and *pyr 3a* recovered with its pyrimidine requirement unchanged.

The effect of pyr 3a on citrulline utilization.—As may be seen from data in table 4, the utilization of citrulline by *orn 1* and *2* and *cit 1* appears to be influenced by the presence of the *pyr 3a* gene. This effect is more marked in the case of *pyr 3a-cit 1*, which, in the presence of cytidine, gave a very meager response to citrulline at any level tested, whereas *pyr 3a-orn 1* and *pyr 3a-orn 2* appear to have merely increased requirements. There is no effect in the case of *pyr 3a-orn 3* nor has there been observed any effect upon ornithine utilization by *orn 1*, *2* and *3* nor upon arginine utilization by any of these strains. (*Pyr 3a-cit 2* has not yet been obtained because of rather close linkage which exists between these two loci.) Cytidine has not been found to affect the utilization of citrulline by the single *orn* and *cit* mutants, nor does citrulline interfere with cytidine utilization by *pyr 3a*.

It is of interest that, as table 4 shows, dry weights from the *pyr 3a-orn* double mutants cultured on cytidine plus citrulline were in some cases as much as twice that given by *pyr 3a* alone on the same amounts of cytidine and citrulline. This again demonstrates the partial suppression of the pyrimidine requirement in these double mutants.

Other recombinants.—There are known at present, in addition to *pyr 3a*, three non-allelic pyrimidineless mutants, *pyr 1* (263),[1, 10] *pyr 2* (38502),[1, 10] and *pyr 4* (36601).[11] It has already been reported that *pyr 1* and *2* are not suppressed by *s* and this has been found to be true also of *pyr* 4. These mutants, and *pyr 3a* as well, are inhibited by arginine when uracil is the only exogenous source of pyrimidine, but there is no inhibition if cytidine or uridine is added. No greater than half inhibition has been observed at any level of arginine tested, and there is no release by lysine. In double

mutants with *orn 3*, *pyr 1*, *2* and *4* were not suppressed and, in the presence of added cytidine, did not affect the behavior of *orn 3*.

The double mutants of *pyr 3a* with *prol* 2 and *3*, when tested in flasks, behaved as would be predicted from the properties of the single mutants. No effect of either mutant gene upon the requirement introduced by the other was observed. Some isolates of *pyr 3a-prol 1* behaved is this manner, but others, which grew on complete medium, failed to grow when supplied cytidine and proline. This has not been investigated further.

It has been reported[2] that 36703, a mutant which uses only arginine[3] showed no interaction with *pyr 3a* or *s*. More recently a histidineless mutant, C84[6] was tested, with the same result. In linkage tests[12] *pyr 3a* or one of its possible alleles, has been crossed to mutants requiring inositol, pyridoxin, pantothenic acid, choline, adenine, phenylalanine, isoleucine + valine and tryptophan, respectively, and no suppression of the pyrimidine requirement was observed.

SUMMARY OF SINGLE MUTANTS AND RECOMBINANTS

Single Mutants

prol 1	slight growth on minimal—uses proline.
prol 2, *prol 3*	grow slowly on minimal—use proline, ornithine, citrulline or arginine.
orn 1, *orn 2*	no growth on minimal—use ornithine, citrulline or arginine.
orn 3	grows slowly on minimal—uses ornithine, citrulline or arginine.
cit 1, *cit 2*	grow slowly on minimal—use citrulline or arginine.
pyr 1	slight growth on minimal—requires pyrimidine.
pyr 2	no growth on minimal at 35°C.—requires pyrimidine.
pyr 3a, *pyr 4*	no growth on minimal—require pyrimidine.
s	no phenotypic differences from wild type observed.

Recombinants

prol 1-s	no phenotypic differences from *prol 1* observed.
prol 2-s, *prol 3-s*	no phenotypic differences from wild type observed.
orn 1-s, *orn 2-s*, *orn 3-s*, *cit 1-s*, *cit 2-s*	no growth, or very slight growth on minimal—use citrulline or arginine but not ornithine.
pyr 1-s, *pyr 2-s*, *pyr 4-s*	no phenotypic differences from *pyr 1*, *2* and *4* observed.
pyr 3 a-s, *pyr 3a-prol 2-s*, *pyr 3a-prol 3-s*	growth on minimal approximates that of wild type—inhibition by proline, ornithine, citrulline or arginine relieved by lysine.

pyr 3a-prol 2, *pyr 3a-prol 3*	no growth on minimal—require pyrimidine + proline, ornithine, citrulline or arginine.
pyr 3a-orn 1, *pyr 3a-orn 2*	no growth on minimal—grow slowly without added pyrimidine on medium supplemented with ornithine, citrulline or arginine—in presence of cytidine the citrulline requirement is higher than that of *orn 1* or *2*.
pyr 3a-orn 3	indistinguishable from *orn 3* on minimal—grows slowly without pyrimidine on medium supplemented with ornithine, citrulline or arginine—with cytidine present the response to these compounds is like that of *orn 3*.
pyr 3a-cit 1	no growth on minimal or on medium supplemented with cytidine alone. In the presence of cytidine the response to arginine is like that of *cit 1-s*, but there is almost no response to citrulline.
pyr 1-orn 3	slight growth on minimal—uses cytidine + ornithine, citrulline or arginine.
pyr 2-orn 3	no growth on minimal at 35°C.—uses cytidine + ornithine, citrulline or arginine.
pyr 4-orn 3	no growth on minimal—uses cytidine + ornithine, citrulline or arginine.

Discussion.—In order to simplify discussion of the observations presented, a summary of the various interactions involving *pyr 3a* and *s* is given below.

The Effects of the Suppressor of Pyrimidineless:

1. It suppresses *pyr 3a* in the absence of added proline, ornithine, citrulline or arginine, or, in the presence of these compounds if lysine also is added.[1]
2. It suppresses two genetically different mutants, *prol 2* and *3*, which require proline, ornithine, citrulline or arginine.
3. It suppresses the double mutants, *pyr 3a-prol 2* and *pyr 3a-prol 3*, which, in combination with it, respond to proline, ornithine, citrulline, arginine and lysine as does *pyr 3a-s*.
4. It prevents the slow growth characteristic of *orn 3*, *cit 1* and *cit 2* on unsupplemented medium.
5. It prevents the utilization of ornithine by the ornithineless mutants.
6. It interferes with the utilization of α-amino adipic acid by the lysineless mutant, *ly 1* (33933) unless arginine, citrulline or ornithine is added.[2]
7. In combination with *pyr 3a* it prevents utilization of α-amino adipic acid by *ly 1*.[2]
8. In combination with *pyr 3a* it interferes with the utilization of lysine by *ly 3* (4545) unless arginine is added.[2]

Effects of Pyrimidineless 3a:

1. It interferes with the utilization of citrulline by *orn 1* and *2* and almost completely stops citrulline utilization by *cit 1*.
2. It prevents the slow growth characteristic of *cit 1* on medium supplemented with cytidine.
3. It interferes with utilization of α-amino adipic acid by *ly 1* unless a small amount of lysine is added.[2]

Effect of orn 1 and 2 on pyr 3a:

They partially suppress *pyr 3a* on medium supplemented with ornithine, citrulline or arginine.

Effect of orn 3 on pyr 3a:

It suppresses *pyr 3a*, completely on minimal medium and partially on medium supplemented with ornithine, citrulline or arginine.

The pyrimidineless suppressor, then, acts as a suppressor of the recognized phenotypic effects of three genes, but when it is present in combination with any one of seven other mutant genes, it acts as an inhibitor, in the sense that it prevents strains carrying these genes from doing something which they are able to do if *s* is replaced by its "wild type" allele. Except for the difference in the degree of suppression, the "wild type" allele of *s* might be more justly called a suppressor. In any case there appears to be no reason for considering *s* to be in a different category from the other mutant genes involved in the interactions described here, since, for example, *pyr 3a* inhibits and the ornithineless mutants suppress. It is possible that "suppressors" which themselves introduce requirements are as frequent as those which do not, but their detection may be less likely. In cases reported by Zalokar[13] and Wagner and Haddox[14] suppression of one mutant by a second was predicted on the basis of growth responses of the first and this suppression was observed when the double mutant was prepared and tested. The double mutant, involving pantothenicless and tyrosine or phenylalanineless, described by Wagner and Haddox, is perhaps unique in that, although it does not grow normally in the absence of pantothenic acid, it is not stimulated by addition of this growth factor.

It now seems unlikely that *s* takes "over the function of the inactive gene at the (*pyr*) *3a* locus" as was suggested previously.[1] In fact it seems likely that the pyrimidine requirement of *pyr 3a* is not due to an "inactive gene," but rather to the activity of a gene being revised in a way such that, in a given genetic background, a pyrimidine requirement results. The requirement, the suppression of it and the other interactions as well, may result from alterations in the balance of reaction rates under the influence of the various mutant genes. The alterations may be pictured as arising through a variety of means, such as changes in enzyme concentrations or activities which produce changes in substrate or inhibitor levels affecting many reactions. The reactions involved may be related by a common coupled system or intracellular organization which is disturbed or modified in some way by the mutant genes. The variety of interactions observed does not point to any one mechanism as a very probable explanation, but rather, appears to suggest a greater number of possible mechanisms.

When the interactions involving the lysineless mutants were first reported it appeared to the authors that these, together with the observed accumulation of uracil by certain lysineless mutants,[2] indicated a close relationship between lysine and pyrimidine biosynthesis. In view of the further complexities reported here, a direct interrelation no longer seems necessarily indicated.

The shifting of the position of the apparent "genetic block" by modifiers has already been reported by Haskins and Mitchell[8] in a paper dealing with tryptophan-nicotinicless mutants. This phenomenon, which is represented here by the action of *s* and *pyr 3a* on the ornithineless, citrullineless and lysineless mutants, suggests that the relationship between the mutation and the nutritional requirement may not always be as direct as it has sometimes been considered to be. As Haskins and Mitchell[8] have suggested, the assumption that the gene affected by the mutation controls, in a direct fashion, the reaction which, on the basis of the results of growth experiments appears to be blocked, does not seem to be a safe one.

The fact that *pyr 3a* and its possible alleles, *3b* and *3c* are suppressed by *s*, whereas *pyr 1* and *2* are not, was at first considered by the authors to be evidence that *pyr 3a*, *b* and *c* were indeed alleles. At that time suppressors which acted on non-allelic mutants were known in Drosophila[15] and more recently Giles[16] has found that certain non-allelic methionineless mutants of Neurospora have a common suppressor and Lein and Lein[17] have reported that the same is true of three non-allelic acetateless mutants, also of Neurospora. It is, then, abundantly clear that response to the same suppressor cannot be considered as proof of allelism. Suppression by *s* of mutants having different requirements is reminiscent of the cases known in Drosophila[15] in which the same suppressor acts upon mutants which are not only non-allelic but which also have different and seemingly unrelated phenotypic effects.

* Work supported by grants from the Rockefeller Foundation.

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