ture alleles. Practically all members of this class have indispensable functions. These genes may have no role in enzyme synthesis, or they may be remotely involved, through controlling the formation of complex intermediates. A majority of the genes in *N. crassa* are in this class.

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*IMMUNOGENETIC STUDIES OF PSEUDOALLELISM IN DROSOPHILA MELANOGASTER. I. ANTIGENIC EFFECTS OF THE LOZENGE PSEUDOALLELES**

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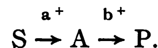
It has been proposed[1, 2] that the phenotypic similarities and position effects of pseudoalleles may be explained by the hypothesis that such loci are concerned with successive steps in a chain of reactions which occurs at the site of the genes in the chromosomes:



$$S \xrightarrow{a^+} A \xrightarrow{b^+} P.$$

Localization could be the result of limited amount or diffusibility of the intermediate A. The sequence of reactions would then proceed to completion only if the wild alleles of the adjacent loci are on the same chromosome, but not if they are on the different members of a pair of homologs.

This hypothesis seems not to be contradicted by position pseudoalleles with morphological effects in Drosophila, and indeed has led to the discovery of pseudoallelism among certain classes of biochemical mutants in Aspergillus.[1, 3, 4] It is a particular form of the "kinetic" hypothesis of

position effect, a hypothesis which attributes position effects to the interactions of products of neighboring genes.[5–7]

Previous immunogenetic studies of Drosophila have been successful in demonstrating the interaction of non-allelic loci in the determination of antigenic specificity.[8–10] Such methods therefore seemed a promising approach to the problems of position pseudoallelism.

Stocks.—The lozenge mutants in *Drosophila melanogaster* have been demonstrated to occur at three closely linked loci on chromosome 1:[11]

$$\underline{\text{sn} \quad 6.7 \quad lz^{BS}0.09\; lz^{46}0.06\; lz^{g} \quad 5.3 \quad \text{v}}$$

The major effects of these mutants are on the eye, and they exhibit the phenotypic characteristics of pseudoalleles including position effects. There are also pleiotropic effects on female fertility, taral claws, pulvilli, and antennae.[12–16]

The three lozenge mutants (BS, 46, and g) were made available to this investigation in stocks provided by M. M. Green. These stocks possessed *X*-chromosomes with one, two, or all three of the mutants together with sn^3 and v, balanced over ClB; sn^3 lz^x v/ClB. For purposes of antigenic comparison it was necessary that the mutants be placed on a coisogenic background. The background chosen was that of the isogenic Oregon R (Series I) stock developed by Jack Schultz. At the time of this derivation, the Oregon R stock had been maintained through sixty-five generations of brother-sister matings since the time that it had been rendered isogenic.

The derivation was accomplished in two steps, the details of which are available from the authors upon request and will be published elsewhere.[16] First, the lozenge segment of each of the *X*-chromosomes mentioned above was inserted into the *X*-chromosome of the Oregon R stock. In each case this was achieved by two successive crossovers between the lz *X*-chromosome and the Oregon R *X*-chromosome, one in the region between sn and lz, and one in the region between lz and v. When this step was completed, Oregon R autosomes and *Y*-chromosome were introduced into each of the lozenge stocks by means of the familiar "dominant marker-crossover inhibitor" technique.

The result of this derivation was, then, a series of eight coisogenic stocks; the Oregon R wild stock, the three single mutant stocks (BS, 46, and g), the three double mutants (BS 46, BS g, and 46 g), and the triple mutant (BS 46 g). Each of the mutant stocks was carried in balanced condition: lz^x/ClB x lz^x. From these, equal numbers of lz^x/lz^x ♀♀ and lz^x ♂♂ could be collected as desired. Heterozygous females were produced, as needed, by crosses between the stocks.

The following qualifications of coisogenicity should be mentioned: (1) Aside from the differences with respect to the lozenge loci, the *X*-chromosomes of the stocks may differ with respect to a segment no more

than 6.7 crossover units long to the left and 5.3 units long to the right of the lozenges. (2) Heterogeneity of autosomes (2 and 3) may have resulted from crossing-over between Oregon R chromosomes and "marker" chromosomes during the derivation, in spite of the multiple inversions present in the latter. (3) The fourth chromosome was not controlled during the derivation. Repeated outcross to Oregon R renders it probable, however, that the mutant stocks possess Oregon R fourth chromosomes. (4) Spontaneous mutation, without visible effect, could not be controlled during or after the derivation.

Immunological Methods.—Flies of desired genotypes were collected from mass cultures, lyophilized, and homogenized in saline. Whole homogenates were used for immunization of rabbits by means of intraperitoneal injection. The homogenates were centrifuged and the supernates used as antigen for absorption of antisera and in precipitin tests of unabsorbed and absorbed antisera. The details of these methods are given in previous publications.[8-10] In previous work, the supernates were ether-extracted

TABLE 1

ANALYSIS OF ANTI-lz^{BS} SERUM

Antiserum, normal serum, and antigen controls all negative

ABSORBED BY	TESTED WITH Ore-R	lz^{BS}	lz^{46}	lz^{g}
...	+++	+++	+++	+++
Ore-R	...	++	++	+
lz^{BS}	...	...	...	...
lz^{46}	...	...	...	...
lz^{g}	...	+	+	...

before use as antigen to avoid non-specific clouding. In this work, ether extraction was not performed and non-specific clouding was avoided by the use of supernates at dilutions above 1:4. Antigen, antiserum, and normal serum controls were included in all tests.

Only qualitative summaries of the results are given in this paper. The full results, which are semiquantitative in nature, are similar to those presented in previous publications.[8-10] They will be supplied by the authors upon request.

Results and Conclusions.—It is convenient to begin the discussion with the results obtained with anti-lz^{BS} serum (antiserum to lz^{BS}/lz^{BS} ♀♀ and lz^{BS}/Y ♂♂ in approximately equal numbers). These results are summarized in table 1.

Unabsorbed anti-lz^{BS} serum reacts with all antigenic preparations, and does so with equal titer. Most of the antigens present in the stocks are therefore similar. Following absorption with Oregon R antigens (prepared from approximately equal numbers of males and females), differences among the stocks begin to appear. The serum no longer reacts with

Oregon R, but still reacts with the antigenic preparations of lz^{BS} (lz^{BS}/lz^{BS} ♀♀ and lz^{BS}/Y ♂♂ in approximately equal numbers), lz^{46} (lz^{46}/lz^{46} ♀♀ and lz^{46}/Y ♂♂), and lz^{g} (lz^{g}/lz^{g} ♀♀ and lz^{g}/Y ♂♂). It appears, therefore, that lz^{BS} contains one or more antigenic components which are not present in Oregon R, but which are shared at least in part with lz^{46} and lz^{g}. On the basis of strength of reaction, lz^{46} appears to be more closely related to lz^{BS} than is lz^{g}.

The specificity of the serum is made evident by its exhaustion by absorption with lz^{BS}. Absorption with lz^{46} yields similar results, indicating that lz^{BS} possesses no antigenic components not also possessed by lz^{46}. Following absorption with lz^{g}, the serum no longer reacts with Oregon R or lz^{g} but still reacts with lz^{BS} and lz^{46}. Thus, lz^{g} does not possess all of the antigenic components which distinguish lz^{BS} and lz^{46} from Oregon R.

These conclusions are confirmed by the results of the analysis of anti-lz^{46} serum (table 2). Again, unabsorbed serum reacts, with equal titer, with all of the antigenic preparations. Absorption by Oregon R eliminates

TABLE 2

ANALYSIS OF ANTI-lz^{46} SERUM

Antiserum, normal serum, and antigen controls all negative

ABSORBED BY	TESTED WITH			
	Ore-R	lz^{BS}	lz^{46}	lz^{g}
...	+++	+++	+++	+++
Ore-R	...	++	++	+
lz^{BS}	...	...	...	...
lz^{46}	...	...	...	...
lz^{g}	...	+	+	...

reactivity with Oregon R but does not completely remove antibodies capable of reacting with lz^{BS}, lz^{46}, and lz^{g}. It therefore appears that lz^{46} possesses one or more antigenic components not possessed by Oregon R, and that at least part of these are also present in lz^{BS} and lz^{g}. Again, a closer relationship between lz^{46} and lz^{BS} than between lz^{46} and lz^{g} is suggested by the differences in strength of reaction.

The serum is completely exhausted by lz^{46} and is therefore specific. It is also exhausted by absorption with lz^{BS}. This result, together with that obtained by absorption of anti-lz^{BS} serum with lz^{46}, argues for the antigenic identity of lz^{BS} and lz^{46}. The results obtained with lz^{g} absorbed serum confirm the conclusion that lz^{g} does not possess all of the antigenic components which distinguish lz^{BS} and lz^{46} from Oregon R. The distribution of antigenic components indicated by the analysis of lz^{46} antiserum is the same as that indicated by lz^{BS} antiserum.

Additional confirmation of this distribution of antigens is obtained from the analysis of anti-lz^{g} serum (table 3). Unabsorbed serum reacts with all of the antigenic preparations. Absorption by Oregon R yields results

which indicate that lz^g possesses one or more antigenic components not present in Oregon R, and that these are also present, at least in part, in lz^{BS} and lz^{46}. Absorption by lz^g demonstrates that the serum is specific. Absorption by lz^{BS} and by lz^{46} yields results which demonstrate that lz^g possesses no antigenic components not also possessed by the other mutants.

The simplest explanation of the results of these analyses is that lz^{BS} and lz^{46} possess two antigens not present in Oregon R, and that lz^g possesses only one of the two. For purposes of convenience the antigen present in all three lozenge mutants will be referred to as L-1. The antigen present in lz^{BS} and lz^{46} will be called L-2.

The single mutants thus possess antigenic components which distinguish them from wild. Does the wild genotype possess antigens distinguishing it from the mutants? To date, the answer is negative. Three Anti-Oregon R sera have been tested, and in each case the mutant antigens were all capable of completely exhausting the serum by absorption.

TABLE 3

ANALYSIS OF ANTI-lz^g SERUM

Antiserum, normal serum, and antigen controls all negative

ABSORBED BY	TESTED WITH Ore-R	lz^{BS}	lz^{46}	lz^g
...	+++	+++	+++	+++
Ore-R	...	+	+	+
lz^{BS}	...	...	...	...
lz^{46}	...	...	...	...
lz^g	...	...	...	...

A test for position effect on antigens is nevertheless possible. Such a position effect, if parallel to that exerted on the eyes, would be evidenced by the presence of L-1, or L-2, or both in repulsion-phase heterozygotes, but their absence in coupling-phase heterozygotes. Alternatively, a position effect would be evidenced by an antigenic difference between contrasting heterozygotes even if neither resembled wild or the mutants.

Such a test has been performed for the heterozygotes involving lz^{BS} and lz^{46}. Repulsion-phase heterozygous females (BS ++/+ 46 +) were produced by matings of lz^{BS} males with lz^{46}/C1B females. Coupling-phase heterozygous females (BS 46 +/+++) were produced by matings of $lz^{BS}lz^{46}$ males with Oregon R females. Reciprocal crosses were not performed, but there is no evidence that a maternal effect is associated with the lozenges, either with respect to their various phenotypic effects or with respect to the position effect on eyes.

The results of the analysis of anti-BS ++/+ 46 + serum are contained in Table 4. Tests were performed only with lz^{BS} among the mutants, since it possesses both L-1 and L-2.

The unabsorbed serum reacts with all of the antigenic preparations. Absorption with Oregon R removes reactivity with Oregon R and lz^{BS}, but antibodies remain which react with BS ++/+ 46 + and BS 46 +/+++. Thus, BS ++/+ 46 + possesses one or more antigenic components not present in Oregon R, and none of these can be L-1 or L-2 since the ab-

TABLE 4

ANALYSIS OF ANTI-lz^{BS}/lz^{46} SERUM

Antiserum, normal serum, and antigen controls all normal

ABSORBED BY	Tested with			
	Ore-R	lz^{BS}	BS + +/+ 46 +	BS 46 +/+ + +
...	+++	+++	+++	+++
Ore-R	...	...	++	++
lz^{BS}	...	...	++	++
BS + +/+ 46 +	...	...	...	...
BS 46 +/+ + +	...	...	...	...

sorbed serum does not react with lz^{BS}. Furthermore, the coupling-phase heterozygote shares at least part of this specific fraction. These same conclusions are supported by the absorption with lz^{BS}, and it is also evident that Oregon R shares no antigens with BS ++/+ 46 + that are not also present in lz^{BS}. Absorption with BS ++/+ 46 + completely exhausts the serum, as does absorption with BS 46 +/+++. The serum is therefore specific, and the repulsion-phase heterozygote possesses no antigens not also present in BS 46 +/+++.

TABLE 5

ANALYSIS OF ANTI-$lz^{BS}lz^{46}$/+ SERUM

Antiserum, normal serum, and antigen controls all negative

ABSORBED BY	Tested with			
	Ore-R	lz^{46}	BS + +/+ 46 +	BS 46 +/+ + +
...	+++	+++	+++	+++
Ore-R	...	...	++	++
lz^{46}	...	...	++	++
BS + +/+ 46 +	...	...	...	...
BS 46 +/+++	...	...	...	...

These conclusions are supported by the analysis of BS 46 +/+++ antiserum (table 5). In these tests only lz^{46} among the mutants was used. It will be recalled that it, like lz^{BS}, possesses both L-1 and L-2.

The results of the absorptions with Oregon R and lz^{46} permit the conclusion that the coupling-phase heterozygote possesses an antigenic frac-

tion not present in either Oregon R or lz^{46}, and shared, at least in part, with the repulsion heterozygote. The absorption with BS ++/+ 46 +, in that it results in complete inactivation, leads to the conclusion that BS 46 +/+++ possesses no components not also present in BS ++/+ 46 +. Since the reverse was demonstrated in the previous analysis of anti-BS 46 +/+++ serum, it may be concluded that the two heterozygotes possess qualitatively identical antigenic structures.

Thus, it appears that the two heterozygotes are indistinguishable with regard to antigenic structure. There is no position effect. However, they both possess a new component, L-3, not found in any of the other genotypes, and both lack L-1 and L-2.

L-3 could be attributed either to heterozygosity itself or to the fact that the BS ++/+ 46 + and BS 46 +/+++ antigenic preparations contain only females. It will be recalled that all of the other preparations contain both males and females. The latter interpretation is rendered unlikely by the inability of the other antigens to exhaust the anti-heterozygote

TABLE 6

SUMMARY OF ANTIGENIC DISTRIBUTION

GENOTYPE	ANTIGENIC COMPONENTS L-1	L-2	L-3	Residual
Wild	...	...	...	+
lz^{BS}	+	+	...	+
lz^{46}	+	+	...	+
lz^{g}	+	...	...	+
lz^{BS}/lz^{46}	...	...	+	+
$lz^{BS}lz^{46}/+$	...	...	+	+

sera even at the highest concentrations used, but has been subjected to independent tests.

In these tests, anti-BS 46 +/+++ serum was absorbed separately with lz^{46} males and with lz^{46}/lz^{46} females. In neither case was the serum exhausted of its reactivity with BS 46 +/+++ and BS ++/+ 46 +. Thus L-3 is not a "female-specific" component, but rather a component specific to the heterozygotes.

The antigenic relationships of the analyzed stocks are summarized in table 6.

Discussion.—Pseudoallelic mutants are mutants which exhibit the phenotypic characteristics of allelism (similar or identical phenotypes in homozygotes and interaction in heterozygotes), but which exhibit a low order of recombination in breeding experiments.[17, 18] The usual interpretation given to such observations is that such mutants occupy adjacent, duplicate loci on homologous chromosomes. An alternative interpretation suggests that such mutants are minute rearrangements in a chromosome segment not separable into discreet loci.[19] Such an interpretation is

vitiated in part by the small number of loci identified in pseudoallelic series (cattle blood-antigens[20] are not a case in point, primarily because of the lack of critical breeding data), but more significantly by its use of phenotypic and physiological observations as a basis for conclusions regarding transmission phenomena. The practice of operationalism, as defined by Bridgman[21] and used by Wright,[22] reconciles the apparent contradictions between the physiological and transmission aspects of pseudoallelism.

It has been suggested, both on cytological and physiological grounds, that new genetic material may arise by a process of genic replication along the chromosome, accompanied or followed by complementary differentiation, and resulting in distinct genes separable by crossing-over and chromosome breakage.[1, 23] Pseudoalleles presumably represent an intermediate stage in this process. The present work lends support to such a concept, since in terms of antigenic effect the locus of lz^g appears to be differentiated partially from that of lz^{BS} and lz^{46}. Similar differentiation has been observed for the effects of these loci on claw development.[16]

The physiological differentiation of pseudoalleles could take three forms: (1) With slight changes in substrate specificity pseudoallelic loci could become associated with successive steps in a sequence of reactions, (2) they could be modified in such a manner as to convert the same substrate into different products, or (3) each locus could be concerned with the transformation of two or more substrates, and differentiation could take the form of a gradual shift in "substrate spectrum." The first possibility is incorporated in the hypothesis of position pseudoallelism proposed by Lewis and by Pontecorvo. The second and third possibilities are similar to models proposed by Wright for allelic differences.[24] With suitable assumptions regarding amorphism or neomorphism of the mutants, any of these three models can explain the aspects of the present data concerned with homozygotes. Antigenic analysis of the unexamined genotypes should provide definite information.

The most striking feature of the present work is concerned with the heterozygotes, i.e., with the absence of a position effect and the appearance of a distinctive antigenic component. The latter phenomenon implies some sort of interallelic interaction, imposed upon the mechanisms of interlocus interaction suggested in the preceding paragraph. The interaction could involve lz^{BS} and its wild allele, or lz^{46} and its wild allele, or both, and analysis of other genotypes should clarify the situation. Whatever alternative is correct, the distinctive component provides another example of genic interaction in antigen production, and implies an interchromosomal mechanism in contradistinction to the intrachromosomal sequence of reactions proposed in the Lewis-Pontecorvo hypothesis.

As for the disparity between the position effect on eyes, a similar position effect on claws,[16] and the lack of position effect on antigens, it is only

possible to make two suggestions: (*a*) The disparity may involve "genuine" pleiotropy, or (*b*) the effects on eyes and claws may involve gene action at a different level than the antigenic effects.

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*A RELATIONSHIP OF HOMOCYSTEINE METABOLISM TO THIAMIN, SERINE, AND ADENINE BIOSYNTHESIS IN A MUTANT STRAIN OF NEUROSPORA**

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A growth inhibition of a strain of *Neurospora crassa* (UT77a) by L-threonine has been described in a previous communication.[1] Growth of UT77a on minimal medium at 35°C. is equivalent to that of wild type strains, but in the presence of inhibitory concentrations of threonine the amino acids methionine, homocysteine, or homoserine must be included