PHENOGENETICS OF THE LOZENGE LOCI IN *DROSOPHILA MELANOGASTER*.

II. GENETICS OF LOZENGE-KRIVSHENKO. (lz^k)

M. M. GREEN

Department of Genetics, University of California, Davis

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PREVIOUS studies of the sex-linked, recessive lozenge (lz) eye mutants in *Drosophila melanogaster* established that each of 18 independent mutants could be assigned to one of three recombinationally discrete loci (GREEN and GREEN 1949, 1956). On phenotypic grounds all but two of these mutants— lz^{49h} and lz^{50e} —fulfilled the accepted criteria of allelism. The two exceptions were distinctive in that homozygous females possessed both spermathecae, invariably missing in typical lz females, and normal tarsal claws, invariably abnormally reduced in lz mutants. In addition a complementary, near wild-type phenotype resulted when the atypical mutants were compounded to any of the typical lz mutants. Despite these phenotypic differences, the two atypical mutants were recombinationally localized to the left or spectacle (spe) locus, and extensive genetic tests failed to demonstrate that they were separable from this locus.

In *Drosophila Inform. Serv.* 30, 1956, DR. J. KRIVSHENKO reported finding a new spontaneous mutant of unusual interest, which he described as almondex-55 (amx^{55}). This mutant was of particular interest because it had been previously shown that the original amx mutant whose phenotype superficially resembles that of lz , represented a tightly linked but nonallelic locus (GREEN and GREEN 1956). While homozygous amx^{55} females are fertile, those of the original amx are sterile. Although KRIVSHENKO localized the new mutant to the lz region, he designated it an amx allele because it was normal when compounded to lz . Lacking the original amx , he could not apply the phenotypic test for allelism.

These facts suggested that amx^{55} was indeed peculiar and merited further study if for no other reason than to decide whether it is an amx or lz mutant. The results of this investigation are the subject of this report. Sincere thanks are due DR. KRIVSHENKO for his generosity in making amx^{55} available for study.

Phenotypic interactions: In phenotype amx^{55} (from hereon designated lz^k) is distinguishable from both amx and any of the known lz mutants. Thus lz^k males have narrowed, moderately rough eyes and normal tarsal claws. The facet derangement is visibly distinctive from that of amx but not obviously different from a number of "rough" eye mutants including some lz mutants. Homozygous lz^k females have normal genitalia, normal tarsal claws and have eyes distinctly more normal than those of lz^k males often being inseparable from wild type. These females are phenotypically separable either on genitalia phenotype and/or eye phenotype from any amx or lz females. In addition lz^k clearly does not manifest

dosage compensation since the eye phenotype of homozygous females is clearly more normal than that of hemizygous males. The absence of dosage compensation was corroborated by comparing the phenotypes associated with the three genotypes lz^k/lz^k , lz^k/lz deficiency and lz^k/Y . As anticipated for mutants which do not manifest dosage compensation, lz^k/lz deficiency females were phenotypically equivalent to lz^k males and unequivocally more abnormal than lz^k/lz^k females. This phenotypic distinction was observed where any one of three independent lz deficiencies was employed. The tests with the lz deficiencies confirm KRIVSHENKO's conclusion on the genetic location of lz^k .

The phenotypic interactions of lz^k were studied in compounds with *amx*, 12 *lz* mutants, three *lz* deficiencies and one *lz* inversion. The eye structure, tarsal claws and spermathecae of heterozygous females for each genotype were studied and compared. The tarsal claws of all heterozygous females studied were normal in appearance irrespective of the *lz* mutant involved. As regards eye structure and spermathecae, the results have been summarized in Table 1. Some comments are in order. It is recognized that a subjective factor is unavoidably inherent in all visual assessments of eye phenotype. Therefore wherever possible phenotypes were compared side by side. Nonetheless, it should be understood that although the listing in Table 1 implies discontinuous phenotypic classes, there is, in fact, some overlapping because of slight and subtle differences which cannot be properly expressed. However, there is sharp separability between classes at the ex-

TABLE 1
Eye and genitalia phenotypes of females lz^k/lz mutant

Compound with <i>lz</i> mutant	Eye phenotype*	Number spermathecae/female			
		0	1	2	3
1. lz^8	±	2	2	21	..
lz^{48c}	+	36	15	1	..
lz^{36}	+	27	2	1	..
lz^{3N}	0-±	..	..	all	..
lz^{34k}	±	..	..	all	..
lz^{BS}	±	41	2	22	..
lz^{50e}	0	..	..	all	..
2. <i>lz</i>	±	..	..	33	13
lz^{48f}	+	..	..	all	..
lz^{46}	±	..	..	all	..
3. lz^g	+	..	..	all	..
lz^{y4}	++	all	..	..	..
lz^3	+++	all	..	..	..
4. $In(1)lz^{sB}$	±	15	14	7	..
$Df(1)lz$	+	..	..	all	..
5. <i>amx</i>	0	..	..	all	..

* Arbitrary eye phenotype scale:

0=wild type.

±=equivalent to homozygous lz^k females.

+=equivalent to lz^k males.

++=rougher and narrower than lz^k males.

+++ =rougher and narrower than ++ class.

tremes. Thus flies in class 0 are always distinguishable from those in classes +, ++ or +++, but not necessarily so from $\pm$. Similarly some individuals in class + may be as extreme as those of class ++. In making these groupings an attempt has been made to represent a composite phenotypic picture. For each genotype, a minimum of 25 females was dissected and their spermathecal phenotype determined. This number is denoted by "all" in Table 1. Where variable spermathecal phenotypes were encountered usually more than 25 females were dissected. Although quantitative studies were not made, there appears to be a correlation between the development of the spermathecae and the ovaries, and fertility. Those females possessing normal spermathecae manifested normal fertility, while those lacking spermathecae exhibited reduced fertility. (For similar behavior in lz^{s7} see ANDERSON 1945.) The data in Table 1 have been separated into five groups depending upon the lz mutants employed: group 1, mutants all localized to the *spe* or left locus; group 2, mutants localized to the lz or middle locus; group 3, mutants localized to the *gly* or right locus; group 4, chromosomal rearrangements and group 5, *amx*. It will be noted that the compound amx/lz^k is wild type in all respects and it is concluded that lz^k and *amx* are not alleles.

The data listed in Table 1 show that it is not possible on the basis of phenotype to draw hard and fast conclusions on the allelic relationships of lz^k . Each compound must be judged by itself. The eye and female genitalia phenotypes which result when lz^k is compounded with lz^{y4} or lz^s are compatible with the conclusion that lz^k is a lozenge mutant. On the other hand the compound with lz^g known to be allelic to lz^{y4} and lz^s does not support this conclusion. The compounds with the mutants lz , lz^{46} and lz^{48f} manifest complementary phenotype and that with lz is especially interesting because a kind of "super" complementation was observed. In repeated crosses about 25 percent of such females possessed three spermathecae rather than the normal two. In Figure 1 the spermathecal condition in lz^k/lz females is illustrated. The compounds with the *spe* mutants present a puzzling and certainly nonlinear picture about which detailed comment is necessary. There appears to be no over-all relationship between the phenotype of the *spe* mutant and of its compound with lz^k . Thus lz^{sN} whose eye phenotype is more abnormal than that of lz^{s4k} or lz^{sB} produces a more normal compound with lz^k than do these less extreme mutants. It is not surprising that the compound lz^{50e}/lz^k is wild type since lz^{50e} , as noted, produces a complementary phenotype with all typical lz mutants tested.

The phenotypic interactions of lz^s , lz^{s6} , lz^{48c} and lz^{sB} are of more than passing interest since all produce the most extreme phenotypic departures from wild-type characteristic of the lozenge mutants. Insofar as is known, no objective way of separating these mutants has been described. Since lz^{sB} is known to be associated with an inversion, it is genetically separable from the others. A comparison of the eye and spermathecal phenotypes of the three compounds with lz^k shows that they are not identical and fall into three distinctive groups. Thus the compound with lz^{s6} results in the most extreme phenotype, the compound with lz^s

FIGURE 1.—Spermathecae of Lz/lz^k females showing (a) individual with normal pair; (b) individual with three.

the least extreme and that with Lz^{48c} falls between the two. These observations suggest that Lz^s , Lz^{36} and Lz^{48c} known to occupy identical loci and seemingly phenotypically identical, are, in fact, distinctive, nonidentical mutations.

Recombination analysis: Because of the unpredictable phenotypic interactions of Lz^k in compound with the several Lz mutants, the determination of the linkage relations of this mutant was of paramount importance. Previous studies had shown that where crossing occurs between two Lz mutants, two complementary products are recoverable: one is Lz^+ , wild type in all respects carrying neither mutant and the second Lz^s -like, simulating the greatest phenotypic departure from wild type and carrying the two Lz mutants coupled to the same X chromosome. If, despite its phenotype, Lz^k is a genuine lozenge mutant which permits free recombination in its genetic environs, these recombination products manifesting precisely the phenotypes indicated are expected among the progeny of females heterozygous for Lz^k and a nonallelic Lz mutant.

Initially crossing over between Lz^k and Lz^{46} was sought since Lz^{46} was assigned to the middle or Lz locus (GREEN and GREEN 1949). Females of the genotype $Lz^k/sn^3 Lz^{46} v$ (sn^3 = singed-3 bristles, v = vermilion eye color) were obtained and crossovers sought among their male progeny. As noted in Table 2, three exceptions were recovered: one male sn^3 but otherwise wild type and two males Lz^s -like v . On the basis of the markers it may be presumed that the exceptions are complementary crossovers. It should be noted that the exceptional males recovered are precisely those expected if Lz^k is localized to the left of Lz^{46} . Since Lz^k on occasion overlaps wild type, the $sn^3 Lz^+$ males were crossed to a deficiency of Lz to make certain that it was indeed a reversion to wild type. The resultant heterozy-

TABLE 2

Crossing over relationships of lz^k

Genotype parental females	Phenotype exceptional males	Total males scored
$sn^s lz^{46} v/lz^k$	1 ♂ $sn^s lz^+$, 2 ♂ lz^s -like v	12,666
$sn^s lz^{BS} v/lz^k$	1 ♂ $lz^+ v$, 1 ♂ $sn^s lz^s$ -like 1 ♂ lz^s -like	12,595

gote proved to be wild type demonstrating that the $sn^s lz^+$ exception is genuinely lz^+ . In phenotype the lz^s -like exceptions simulated that of the mutant lz^s except that the reduction in amount of red eye pigment was not quite as great. Characteristically, the red pigment in lz^s flies is confined to a thin, peripheral rim along the perimeter of the eye. The lz^s -like flies had a slightly broader rim. From previous experience it can be assumed that the lz^s -like exceptions are of the genotype $lz^k lz^{46} v$. This was proved by obtaining females of the genotype lz^s -like v/sn^s and seeking $sn^s lz^{46} v$ and lz^k males among their progeny. As anticipated two $sn^s lz^{46} v$ and one lz^k males were recovered among ca. 15,000 individuals.

Since lz^k has its locus to the left of lz^{46} it was tested for allelism to lz^{BS} similarly localized to the left of lz^{46} . Females of the genotype $sn^s lz^{BS} v/lz^k$ were obtained and their male progeny scored. Unexpectedly, three male exceptions were obtained as indicated in Table 2: one $lz^+ v$, one $sn^s lz^s$ -like, and one lz^s -like carrying neither marker. The first two exceptions are readily explained on the assumption that lz^k has its locus to the right of lz^{BS} and therefore at a new locus, between lz^{BS} and lz^{46} . In this location, crossing over would produce exceptions exactly as recovered. To explain the third exception it is necessary to assume that double crossing over took place with one crossover occurring between lz^k and lz^{BS} and the second between lz^{BS} and sn^s . Both lz^s -like exceptions should carry both lz^{BS} and lz^k in coupling. This was proved in the usual manner. In the case of the lz^s -like exception without markers, one $lz^{BS}v$ and two $sn^s lz^k$ males were found among 12,635 male progeny of females whose genotype was lz^s -like/ $sn^s v$. Where the $sn^s lz^s$ -like exception was tested, one $sn^s lz^{BS} v$ male was detected among 10,040 male offspring of females $sn^s lz^s$ -like/ v .

There can be no doubt that lz^k represents a new lz locus situated roughly equidistant between lz^{BS} and lz^{46} . Additional crosses where lz^k was tested for recombination with lz^{s4} and lz^{sN} allelic to lz^{BS} and with lz allelic to lz^{46} verified in all details the results reported for lz^{BS} and lz^{46} and need not be elaborated here.

DISCUSSION

Since lz^k manifests neither the altered female genitalia, nor the striking altered eye phenotype, nor the reduced tarsal claws phenotypes so characteristic of the lozenge mutants, on what grounds should it be classified a lozenge mutant? The one fact favoring this classification is the lz^s -like phenotype characteristic of individuals whose genotype consists of lz^k coupled to the X chromosome with a typical lozenge mutant. The genotypes $lz^{BS} lz^k$, $lz^k lz^{46}$, $lz^{s4} lz^k$ and $lz^k lz$ all have

been obtained and without exception all manifest eye, genitalia and tarsal phenotypes classifiable as lz^s -like and therefore more extreme than that of either mutant. This observation parallels precisely earlier observations with comparable coupling genotypes of typical lozenge mutants (GREEN and GREEN 1949, 1956). On this basis lz^k cannot be considered an *amx* mutant since it has already been demonstrated that the coupled genotype *amx lz^g* does not produce the characteristic lz^s -like phenotype (GREEN and GREEN 1956). In addition, it should be noted that the phenotypes of females lz^k/lz^s and lz^k/lz^{y4} are, except for the lack of altered tarsal claws, those expected if lz^k is considered an *lz* mutant.

In at least two ways, the phenotypic interactions of lz^k with other lozenge mutants are at variance with the general experience with allelic and pseudoallelic mutants. One difference is the relationship between complementation and the spatial location of the mutants. The experience with *Neurospora* indicates that, with rare exceptions, there is a high correlation between complementation and the spatial location of independent mutants (GILES 1959). Complementation is greatest between mutants which are recombinationally far apart and least or fails to occur between mutants which are recombinationally close together. In the case of lz^k the situation is reversed. Complementary phenotypes are observed among compounds with mutants which are comparatively closely linked to lz^k (e.g. *lz*, lz^{46}) while the most mutant (noncomplementary) phenotypes are found in compounds with mutants whose loci are more distant (e.g. lz^s and lz^{y4}). From this operational point of view, lz^k is an exception to the concept of the cistron. If the aforementioned phenotypic interactions are applied to determine the cistronic relationships of the several lozenge mutants, it would be concluded that lz^k is not included in the same cistron as mutants proximal to it but is part of a cistron which includes more distally located mutants. From the viewpoint of the gene as a functional unit this means that there occur functionally unrelated sites which separate or disturb the integrity of the functionally related elements. While there is no *a priori* reason why a genetic arrangement of this sort should not exist, its existence is not predicted from the cistron concept. The general application of this concept is therefore open to serious reservations.

The phenotypic interactions of lz^k within a group of alleles also fail to fulfill expectations. In general the phenotype of a compound is correlated with the phenotypic alteration associated with the individual mutants being studied; the more extreme the phenotypes of the mutants, the more extreme the phenotypes of the compounds. Among the compounds of lz^k with the alleles lz^g , lz^{y4} and lz^s this rule holds true for in this group lz^g causes the least drastic phenotypic change, lz^s the greatest change. Their compounds with lz^k follow the same phenotypic order. However, among the mutants of the left locus at least two mutants provide clear-cut exceptions. Thus lz^{sN} is phenotypically a far more abnormal allele than is lz^{BS} yet their compounds with lz^k are reversed.

These facts re-emphasize the assertion that the mutant phenotype, except at the molecular level, is not a reliable criterion for describing either the functional or spatial properties of independent mutations. Conclusions based on phenotype are at best presumptive and should invariably be recognized as only a first step

in describing the properties of any mutated gene. To draw conclusions on gene function solely from phenotypic interactions is to indulge in sophistry.

The fact that the mutants lz^s , lz^{36} and lz^{4sc} , genetically and phenotypically indistinguishable even after detailed morphological analysis (CLAYTON 1954a,b) can be separated from one another by virtue of their compounds with lz^k has important implications for the nature of the mutation event. These observations illustrate that as varied and more sensitive methods of phenotypic analysis are brought into play, presumed recurrences of the same mutation can often be shown to be nonidentical. The extreme sensitivity of immunological methods as applied to blood groups, especially in cattle (STORMONT 1959), suggests that an almost unlimited number of nonidentical alleles can occur at one locus. Comparable findings have been made where self-sterility alleles in plants have been similarly studied (EMERSON 1939). There is therefore reason to believe that an equivalent situation can exist at each locus of a pseudoallelic array, the demonstration being predicated only on finding sensitive phenotypic indicators of nonidentity. This suggests that within a recombinationally indivisible unit (the locus!) an extremely large number of discrete mutational events can occur, each phenotypically separable from the others. It suggests further that identical mutational events rarely recur or recur less frequently than nonidentical mutations. What are the dimensions and organization of a locus in this view? It is, at present, not possible to precisely equate minimum genetic size as measured in *Drosophila* and other higher organisms by crossing over and DNA composition. As a working hypothesis it seems that the *Drosophila* locus finds its counterpart in the cistron of the virus and bacterium. This is to say that the recombinationally indivisible unit of the higher organism is equivalent to a microbial unit which is divisible by the process of microbial recombination. A genetical situation of this type can have meaning only if recombination by crossing over in higher organisms and recombination in viruses and bacteria are not identical events. These are on operational grounds good and ample reasons for believing that the recombination events genuinely are different.

SUMMARY

1. A genetic analysis of the mutant lz^k is presented and data submitted to show that it represents a new lozenge locus localized between the previously described left and middle loci.

2. The phenotypic interactions of lz^k and other lz mutants are variable. Compounds with complementary phenotypes occur with mutants proximal to lz^k and noncomplementary phenotype with mutants more distally located.

3. The bearing these findings have on the organization of the genetic material is discussed.

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