HYPOMORPHIC LETHAL MUTATIONS AND THEIR IMPLICATIONS FOR THE INTERPRETATION OF LETHAL COMPLEMENTATION STUDIES IN DROSOPHILA

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ABSTRACT

In a small region of the *X* chromosome of *Drosophila melanogaster*, we have found that a third of the mutations that appear to act as lethals in segmental haploids are viable in homozygous mutant individuals. These viable mutations fall into four complementation groups. The most reasonable explanation of these mutations is that they are a subset of functionally hypomorphic alleles of essential genes: hypomorphic mutations with activity levels above a threshold required for survival, but below twice that level, should behave in this manner. We refer to these mutations as "haplo-specific lethal mutations." In studies of autosomal lethals, haplo-specific lethal mutations can be included in lethal complementation tests without being identified as such. Accidental inclusion of disguised haplo-specific lethals in autosomal complementation tests will generate spurious examples of interallelic complementation.

PUTATIVE lethal mutations of *Drosophila melanogaster*, defined by their exposure by a deficiency, may or may not act as lethals in homozygous or hemizygous conditions (WELSHONS 1971; LEFEVRE and JOHNSON 1973; SANDLER 1977; JOHNSON, WOLOSHYN and NASH 1979; BELYAEVA *et al.* 1980). The probable explanation of this phenomenon involves a trivial extension of MULLER's (1932) definition of hypomorphy to essential genes: the more extreme expression in the hypomorph/deficiency heterozygote in this case results in death, whereas the homozygote (or hemizygote) exhibits sufficient activity to allow survival. The frequency with which this class of mutation is found would be expected to be locus dependent and, in the extreme case, it is possible that the mutant homozygote would exhibit wild-type viability (see DISCUSSION for details).

In the present paper we report experiments in which hypomorphs of this particular kind were selected, along with fully lethal mutations. The hypomorphs are relatively common.

Complementation tests with recessive lethal mutations have been used commonly in recent years to probe genetic organization. Primary emphasis has been placed on the inherently simple patterns of complementation found (JUDD, SHEN and KAUFMAN 1972). However, numerous cases of interallelic (intragenic) complementation have been described, including some instances of

"complex" genes. Sufficient explanations of interallelic complementation are available from the literature. However, hypomorphic mutations of the type described have never been considered in the context of complementation tests; under special conditions, these mutations can lead to results that could be interpreted spuriously as interallelic complementation or extra lethal complementation groups.

MATERIALS AND METHODS

Culture methods have been described previously (JOHNSON, WOLOSHYN and NASH 1979). All cultures were reared at 25°. For explanations of *D. melanogaster* stocks not otherwise described, see LINDSLEY and GRELL (1968).

Males from a *y cv v f* stock were treated with ethyl methanesulfonate (6–16 mM) according to LEWIS and BACHER (1968) and crossed to attached-*X* females from a *Df(1)v*$^{-74}$*/C(1)DX, y w f bb*$^{-}$*/Y; Dp(1;2)v*$^{+63i}$*/+* stock, provided by G. LEFEVRE JR. 7719 *Dp(1;2)v*$^{+63i}$-bearing male progeny (*y cv f*) were backcrossed individually to *C(1)DX, y w f bb*$^{-}$*/Y; Dp(1;2)v*$^{+63i}$ or +/+ females. *Dp(1;2)v*$^{+63i}$ covers the chromosome segment 9E1 to 10A11 (LEFEVRE 1969) and, therefore, allows recovery of a subset of *X*-linked recessive lethals in this region.

Second-generation cultures with no *Dp(1;2)v*$^{+63i}$-free [*v*] males after 2 or 3 days of eclosion were selected. *Dp(1;2)v*$^{+63i}$-bearing [*v*$^{+}$] males from these cultures were mated to *Df(1)203, y cv ras*$^{-}$ *v f/FM6, y*^{31d}*sc*^{8} *dm B* females. For details of *Df(1)203*, which was induced in a *y cv v f* chromosome, see Figure 1. The latter cross was carried out whether or not the parental culture subsequently produced *Dp(1;2)v*$^{+63i}$-free males.

Third generation cultures were scored to completion, and only cultures that yielded no *y cv v f/Df(1)203, y cv ras*$^{-}$ *v f* females (vermilion-eyed, non-Bar) were retained. These cultures appear to contain recessive lethals that are exposed by the deficiency, and, because they are covered by *Dp(1;2)v*$^{+63i}$, the lethals would be supposed to reside in the overlap region, 9E1-9F13. The third generation culture from each mutation found was used to produce a *mutation*/FM6 stock that was subsequently selected to remove *Dp(1;2)v*$^{+63i}$. A parallel stock, derived from second generation progeny of both sexes (*i.e.*, attached-*X* females and *Dp(1;2)v*$^{+63i}$-bearing males), was established for each mutation. Both stocks were maintained by selection against vermilion individuals when such individuals survived.

Complementation tests were carried out by crossing *y cv mutation*$_1$ *v f*/FM6 females with *y cv mutation*$_2$ *v f/Y; Dp(1;2)v*$^{+63i}$*/+* males. In assessing the viability of the double heterozygotes we compared the frequencies of *Dp(1;2)v*$^{+63i}$-free [*v*] and *Dp(1;2)v*$^{+63i}$-bearing [*v*$^{+}$] females. Deviations from a 1:1 ratio between these classes were identified with a simple chi-square test. The results of these tests are presented in Tables 2 and 3. Comparisons between the ratio of these two classes of doubly heterozygous progeny and the equivalent classes derived from homoallelic crosses employed a 2 × 2 contingency chi-square test. Similar 2 × 2 tests were carried out, when meaningful, on the data in Table 1.

RESULTS

Table 1 shows the results of crosses of *Dp(1;2)v*$^{+63i}$-bearing males to *Df(1)-203*/FM6 females for the 24 lines in which a mutation in the region 9E1-9F13 yielded no *Dp(1;2)v*$^{+63i}$-free, non-Bar females. All 24 mutations were of independent origin. Also shown in Table 1 are the results of crosses between the two separate stocks kept for each mutation. These homoallelic crosses have the same balancer and marker chromosomes as the complementation tests. It can be seen that, in eight crosses, homozygous females survive. In seven of these crosses the equivalent hemizygous males also survive. In the eighth cross (J5) no males were found, but in a larger series of crosses using J5/FM6 females in a complementation study of all 24 mutations, a total of 14 J5/*Y* males

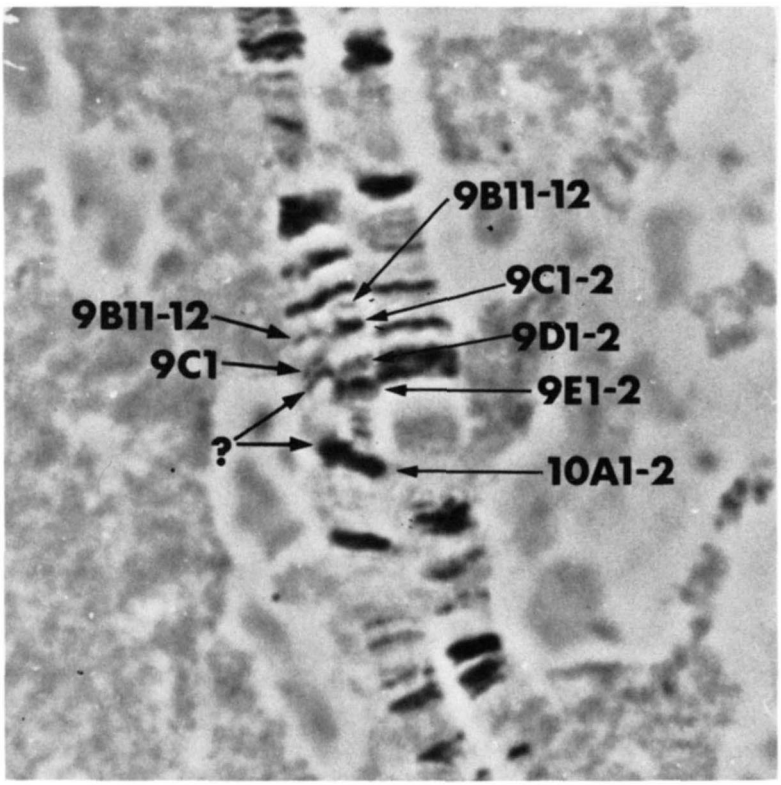

FIGURE 1.—*Df(1)203, ras*$^{-}$/+: The segment 9E1-9F13 appears to be absent from the deficiency chromosome. Material (?) between 9C1 and 10A1-2 is of uncertain origin but may represent an inversion of the region 9C2-9D. This hypothesis would account for the diminution of the 9C1-2 region, the distal augmentation of 10A1-2, and the reduction of the apparent 9C-9D distance, as well as numerous instances of anomalous pairing found in less stretched chromosomes. The aberration was induced by γ-irradiation of a *y cv v f* male. Segment 47 of chromosome *2R* occupies the right-hand portion of the figure. ×1,920.

survived in the absence of *Dp(1;2)v*$^{+63i}$, compared with 1276 J5/*Y*, *Dp(1;2)v*$^{+63i}$ siblings. (This study also confirmed the viability or inviability of *Dp(1;2)v*$^{+63i}$-free males for the remaining 23 mutations). Thus, the eight mutations A15, B4, F16, G16, J5, M14, M27 and Q10 exhibit the behavior ascribed to hypomorphy in the introduction of this article.

The numbers of progeny in the individual crosses in Table 1 are not large; the cross 1 data are generally derived from two cultures with five parents of each sex. However, in all cases except B4 the number of "escapers" in cross 2 represents an increase (at the 5% level of significance) over the number of *mutation/Df* progeny in cross 1. Moreover, all but two (B4 and M14) of the hypomorphs have been shown to behave as lethals against at least one other deficiency, as have all of the fully lethal mutations. This, together with the fact that there is a correlation between the survival of homozygotes and hemizygotes in cross 2, is strong evidence that the phenomenon is real.

In most cases, the hypomorphic mutations behave as semilethals, but, for

TABLE 1

Characterization of a group of 24 putative recessive lethal mutations initially identified as failing to complement with a deficiency

Mutation	Cross 1[a]				Cross 2[b]					
	Females			Males	Females			Males		
	mutation/Df(1)203				*mutation/mutation*			*mutation/Y*		
	mutation/FM6	$Dp(1;2)v^+$ present	$Dp(1;2)v^+$ absent	FM6/*Y*	*mutation*/FM6	$Dp(1;2)v^+$ present	$Dp(1;2)v^+$ absent	FM6/*Y*	$Dp(1;2)v^+$ present	$Dp(1;2)v^+$ absent
A15	*87*	*33*	*0*	*48*	*114*	*65*	*9*	*174*	*54*	*15*
A17	90	29	0	39	114	56	0	109	52	0
B4	*95*	*43*	*0*	*71*	*115*	*60*	*1*	*115*	*51*	*2*
B13	88	32	0	50	103	54	0	97	43	0
D12	24	14	0	17	36	36	0	59	30	0
E20	65	42	0	39	123	40	0	71	56	0
F1	75	30	0	55	86	50	0	67	42	0
F16	*84*	*35*	*0*	*49*	*171*	*67*	*14*	*132*	*79*	*6*
F19	78	25	0	59	75	38	0	68	32	0
F21	35	15	0	31	118	34	0	73	60	0
G4	34	12	0	23	87	35	0	78	43	0
G16	*98*	*51*	*0*	*78*	*80*	*38*	*24*	*75*	*31*	*30*
G19	49	30	0	36	77	33	0	56	29	0
H6	29	19	0	25	163	72	0	137	70	0
H21	76	31	0	62	147	60	0	102	52	0
H22	69	32	0	38	162	80	0	118	63	0
H25	56	22	0	38	84	49	0	80	30	0
J5	*40*	*18*	*0*	*21*	*122*	*33*	*13*	*57*	*64*	*0*
K27	35	18	0	26	123	64	0	102	53	0
M14	*258*	*78*	*0*	*178*	*89*	*49*	*12*	*65*	*47*	*11*
M27	*72*	*35*	*0*	*61*	*67*	*34*	*34*	*34*	*39*	*20*
N23	65	17	0	47	99	41	0	97	37	0
Q10	*114*	*57*	*0*	*88*	*62*	*41*	*25*	*64*	*37*	*36*
Q21	94	42	0	68	59	30	0	74	29	0

Data from "hypomorphs" are shown in italics. The presence/absence of $Dp(1;2)v^{63i}$ was distinguished, using wild-type and vermilion eye color. In cross 1, deficiency hemizygotes do not survive, even in the presence of the duplication.

[a] Cross 1: *Df(1)203, y cv ras⁻ v f*/FM6 females × *mutation/Y*; $Dp(1;2)v^{63i}$/+ males.

[b] Cross 2: *mutation*/FM6 females × *mutation/Y*; $Dp(1;2)v^{63i}$/+ males.

M27 females, and G16 and Q10 males, survival rate is close to that of wild type. When the selection screen is considered, it is not surprising that all develop slowly (data not shown). Because mutations that give a normal development rate in hemizygotes were discarded in the selection screen, it is possible that this class of hypomorphs is underrepresented in our data.

The complete set of complementation tests involving the eight hypomorphic mutations is shown in Table 2. Table 2 includes some data extracted from Table 1. The mutations have been allocated to complementation groups on the basis of data shown either in Table 2 or Table 3. The allocation of mutations A15, B4 and M14 to a single complementation group is based upon the observation that survival of double heterozygotes in all six possible crosses

TABLE 2

Complementation tests involving eight hypomorphic lethal mutations

Female parent (*mutation*/FM6)	Male parent (*mutation*/Y; *Dp(1;2)v*$^{+63i}$/+)							
	A15	B4	M14	G16	M27	Q10	F16	J5
A15	9/65**	3/27**	26/54**	36/38	38/32	39/47	51/40	41/42
B4	4/55**	1/60**	2/24**	32/28	24/27	49/50	35/43	49/34
M14	14/93**	4/48**	12/49**	55/70	59/61	60/40*	29/37	41/30
G16	30/38	16/27	40/43	24/38	32/33	32/68**	34/39	48/36
M27	51/58	34/32	22/36	21/37*	34/34	26/28	34/53*	29/36
Q10	30/39	58/43	52/53	36/50	33/28	25/41*	43/39	31/30
F16	61/63	58/52	46/52	43/43	34/40	22/32	14/67**	88/79
J5	59/50	40/33	58/52	59/47	21/15	30/27	52/41	13/33**

Data represent the numbers of double heterozygotes (or homozygotes in cases in which the mutation is the same in each parent) lacking and carrying *Dp(1;2)v*$^{+63i}$, respectively.

* Deviation from 1:1 at 5% or greater level of significance.

** Deviation from 1:1 at 1% or greater level of significance.

TABLE 3

Complementation test between four hypomorphic lethal mutations (G16, M27, Q10 and F16) and two fully lethal allelic mutations (A17 and H22)

Parent 2	Parent 1 (*mutation*/FM6 female or *mutation*/Y; *Dp(1;2)v*$^{+63i}$/+ male)			
	G16	M27	Q10	F16
A17/FM6 (female)	11/38**	5/34**	1/47**	38/34
A17/Y; *Dp(1;2)v*$^{+63i}$ (male)	0/29**	0/27**	0/26**	41/43
H22/FM6 (female)	0/39**	1/18**	0/37**	60/62
H22/Y; *Dp(1;2)v*$^{+63i}$ (male)	0/29**	0/41**	0/49**	34/40

See Table 2 for definition of superscripts.

is significantly lower than that of the *Dp(1;2)v*$^{+63i}$-bearing internal controls (minimum $\chi^2_1 > 9.8$, $P < 0.01$). Of the 12 possible comparisons between heteroallelic survival and that of the equivalent homoallelic females, one strongly significant difference is found ($\chi^2_1 > 9$, $P < 0.01$), with the double heterozygote (A15/M14) produced by crossing A15/FM6 females to M14/Y; *Dp(1;2)v*$^{+63i}$/+ males being substantially more viable than A15/A15. This probably results from a partial reversion or background modification of M14 before the test was carried out, about 1.5 yr after the initial isolation of the mutation.

Mutations J5 and F16 appear to be unique hypomorphic members of two additional complementation groups. The significantly low survival of F16/M27 double heterozygotes in one of the reciprocal crosses ($\chi^2_1 = 4.1$, $0.05 > P > 0.01$) might be expected as a chance occurrence among the 44 instances in which it is suggested that complementation occurs. This claim, and the even more contentious allocation of mutations G16, M27 and Q10 to a single complementation group, is supported in Table 3, which shows the outcome of

crosses between two allelic lethals, A17 and H22, and G16, M27, Q10 and F16. The exceedingly low survival of G16, M27 and Q10 in double heterozygotes with A17 and H22 suggests that all five mutations belong to the same complementation group. Based upon similar observations (not shown), the mutation J5 has seven lethal alleles. The survival of F16/A17 and F16/H22 at levels not significantly lower than controls appears to confirm that the apparent interaction of F16 and M27 was, indeed, simply a matter of sampling error. F16 has no additional lethal alleles.

The reciprocal differences between crosses involving A17 and its hypomorphic alleles (Table 3, lines 1 and 2) are counterintuitive to expectations for a maternal effect. Their simplest explanation is that the two A17 stocks have diverged somewhat since construction.

Like the relatively low level of survival in double heterozygotes between A15, B4 and M14, the high levels of survival of double heterozygotes between G16, M27 and Q10 should not be interpreted as evidence for interallelic complementation. Although only two of the six heteroallelic crosses yielded significantly fewer double heterozygotes than controls, it is also true that the viability of none of the double heterozygotes differs significantly from either of the parents. This result derives from the fact that the intrinsic viability associated with these particular mutations is high. If it were not for the existence of fully lethal alleles (A17 and H22), there would be considerable doubt as to whether the three haplo-lethal mutations belong to one, two or even three complementation groups. Given that this complementation group involves complicated complementation behavior, as well as the most viable hypomorphs, it is of interest to note that G16, M27 and Q10 act as lethals with four deficiencies against which they have been tested; the aggregate data include 533 *Dp(1;2)v*$^{+63i}$-bearing deficiency heterozygotes compared with no duplication-free flies.

DISCUSSION

Among the sample of 24 apparent lethal mutations ultimately selected by lack of complementation against a deficiency, eight survive as homozygotes and hemizygotes. The hypomorphs are found in four of the eight complementation groups defined by the sample (data not shown). Thus, although the phenomenon is not new and its probable explanation simple, the relatively high frequency of such mutations suggests that their properties and consequences cannot be ignored.

The presumed functional origin of hypomorphic mutations is partial reduction in gene activity. Operationally, hypomorphy is defined by a more extreme phenotype in a mutant/deficiency heterozygote than in the equivalent homozygous mutant (MULLER 1932). When viability is the metric used in defining hypomorphy, the expected results will depend upon the characteristics of the particular essential gene involved.

Figure 2 illustrates three examples of possible relationships between the activity of an essential gene in mutant homozygotes and the probability of survival. It is this relationship that determines the spectrum of mutational types

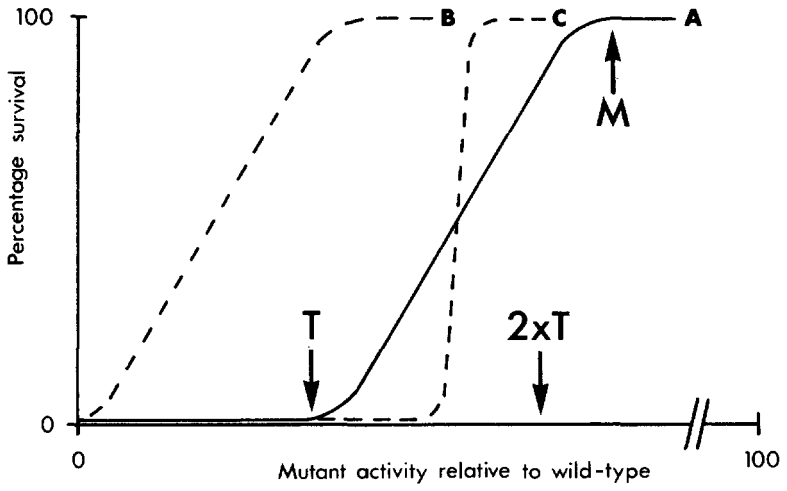

FIGURE 2.—Hypothetical curves showing three possible relationships between gene activity in a series of hypomorphic alleles and the probability of survival of a mutant individual. For curve A, alleles with activity between T and M will be semilethals or subvitals, and those occurring between T and 2×T will be haplo-specific lethals. By modifying the position or slope of the curves (B, C) the spectrum of mutations is changed. Mutant activity, relative to wild type (100), is on a per fly basis, *i.e.*, in a mutant homozygote or a fully dosage-compensated hemizygous male, for an *X*-linked gene.

that can be expected at a given locus. There are two critical points in this relationship: the threshold T, an activity level below which no mutant can survive, and the point M, the lowest activity level that permits maximum survival. These activity levels are indicated for curve A, Figure 2. Mutations yielding activities less than T will be fully lethal, and those between T and M will be semilethal or sublethal. The expected behavior of deficiency heterozygotes (that is, the individuals required for operational definition of hypomorphy) is similarly defined by curves of this kind; survival level of deficiency heterozygotes should relate to half the value of relative mutant activity.

Hypomorphic mutations of the kind described in this paper would have an activity between T and 2T; their deficiency heterozygotes would, thus, fall at or below the threshold for survival (T), providing that the essential gene product is not dosage compensated in a deficiency heterozygote. In the case of *X*-linked mutations, dosage compensation would be expected in the hemizygous males.

If one assumes some standard distribution of mutations in respect to "relative activity," then loci with high threshold (T) values would tend to produce this class of hypomorph more often than those with low T values. Our results, therefore, suggest that it may be common for relatively high levels of activity to be required of essential genes, although none of those included in the study region can have a T value greater than 50%, since *Df(1)203* survives when heterozygous with a wild-type chromosome.

Loci with high T values should also tend to produce fully lethal alleles more commonly and, given equal overall mutability, may appear as mutation hot-

spots. Some fully lethal alleles of these loci will have activity levels greater than zero (but less than T) and, among these, some would be expected to form viable double heterozygotes with some members of the class of hypomorphs described here.

The presumption that all loci yield a spectrum of mutations with a similar distribution of activity is not necessarily correct. Altered spectra could alter the pattern of viability characteristics found at a locus. An extreme case would be a "locus" with two independently functioning, identical genetic elements. If the T value for such a duplicated gene were between half and a quarter of the level produced in the wild type (with its four functional copies), then a double mutant or a deficiency would be required to produce a fully lethal allele, but point mutations abolishing activity of a single gene copy would be lethal against a deficiency.

There is no necessary correlation between the existence of the particular class of hypomorphic lethals and semilethal alleles at a given locus. Curve A is a case in which there *would* be a correlation; many mutations with subnormal viability would be lethal against a deficiency. However, curve B in Figure 2 shows a case in which many semilethals would be found, but the great majority would produce semilethal deficiency heterozygotes. Curve C would yield many mutations with wild-type viability that would be lethal in deficiency heterozygotes. Strictly speaking, such mutations cannot be classified as hypomorphs, because their homozygotes exhibit a wild-type phenotype; they would be more properly described as isoalleles, according to the original definition of the term (STERN 1930). For this reason, we prefer to refer to all mutations of this kind as *haplo-specific lethal mutations*. The novel term also removes the need to specify which class of bona fide hypomorphs is under consideration.

The demonstration by ROBBINS (1983) that, under some conditions of lowered gene activity, viability may be conditioned by maternal genotype, may be relevant to the viability of haplo-specific lethals in combination with an accompanying deficiency. Three of the mutations described (B4, M14 and F16) showed escapers when a deficiency was introduced via the father (data not shown). If maternal effect is a significant factor, then the breeding pattern involved in identification of haplo-specific lethals would modify the frequency with which they are found.

It is relatively simple to interpret the existence of haplo-specific lethal mutations, provided they are recognized. In this situation, their behavior in complementation tests is predictable, although it may be difficult to ascertain allelic relationships in the absence of fully lethal alleles.

There are circumstances under which the existence of a haplo-specific lethal mutation will not be evident. In this study, we eliminated extraneous recessive lethal mutations from the *X* chromosome, except in a small region (10A1-10A11), during the first two generations of the selection procedure. In studies on the autosomes it is difficult to include a similar step, and lethals are generally defined exclusively by their behavior against deficiencies. The effectiveness of ethyl methanesulfonate as a mutagen is so great that many lethals defined in this manner will be accompanied by extraneous, independently in-

duced lethals elsewhere on the same chromosome. Ethyl methanesulfonate is the mutagen used in the majority of studies of lethals in *D. melanogaster*.

The presence of an extraneous, fully lethal mutation in the same chromosome as a haplo-specific lethal mutation would create the impression of full lethality; in addition to the initial test against a deficiency, the chromosome would remain in lethal balance with the "balancer" chromosomal rearrangement used to maintain it in stock. However, it would be most unlikely that two independently isolated chromosomes would contain allelic extraneous lethals. As a result, inclusion of these double mutations in a complementation test would lead to spurious identification of interallelic complementation, for the extraneous lethals would complement, leaving the intrinsic properties of the double heterozygote for the mutations in the study region as the sole determinant of viability. If one (or both) of a pair of allelic mutations is a haplo-specific lethal, this viability can range anywhere between zero and 100%.

Perhaps the best way to illustrate the implications of this conclusion is to reinterpret our own results, ignoring the fact that our mutations are haplo-specific lethals. Following the precedents of SUZUKI and PROCUNIER (1969) and of GAUSZ *et al.* (1979) by assuming that instances of survival represent complementation, the three mutations A15, B4 and M14 would be placed in separate complementation groups (or subgroups, if the criteria for assessment of complementation behavior were stringent). J5 and its lethal alleles would constitute two subgroups of mutations, interconnected by four mutations that show no complementation with either subgroup. Finally, G16, M27 and Q10, together with the lethal alleles A17 and H22, would yield a complex pattern containing three subgroups, two interconnected by one mutation and all three interconnected by another. However, there is no basis in the actual data for proposing any of these subgroups, knowing as we do that some of the mutations are haplo-specific lethal.

In the last decade it has become standard practice to assess genetic organization in Drosophila using lethal complementation tests. As was argued earlier, if such tests involve autosomes, the probable presence of disguised haplo-specific lethal mutations would be expected to suggest partial and even full complementation between allelic mutations.

Haplo-specific lethals certainly occur on the autosomes (SANDLER 1977). From examination of published work it is impossible to demonstrate specific cases in which haplo-specific lethal mutations have led to a false description of complementation. In some work it is clear that this is possible (see, for example, GAUSZ *et al.* 1979; WOODRUFF and ASHBURNER 1979; HILLIKER *et al.* 1980 or LEWIS *et al.* 1980). These studies were all carried out with autosomes with lethals being selected against deficiencies. Studies of lethals selected by other means (SUZUKI and PROCUNIER 1969 and GRACE 1980) are subject to the same objection so long as doubts remain as to whether extraneous lethals are present. In several of these papers it is specifically mentioned that extraneous lethals were not removed; in none of them is it specified that they were.

Such doubts are less pervasive in studies using the *X* chromosome, in which it is much easier to remove extraneous lethals [see LEFEVRE (1981) for refer-

ence to most recent work]. Cases of interallelic complementation are found occasionally in these studies, so it is reasonable to conclude that some of the autosomal cases are also real. ZHIMULEV *et al.* (1981) include an example of a complex locus in region 10A, based exclusively on lethal complementation patterns. Even more spectacular is a pattern described by BELYAEVA *et al.* (1980) in region 2B. These instances imply that the general rule that lethal complementation groups are simple or, at most, involve instances of interallelic complementation attributable to the interaction of the identical subunits of homopolymeric proteins (CRICK and ORGEL 1964) has bona fide exceptions. It should be noted, however, that the phenomenology usually associated with subunit-derived interallelic complementation, the sporadic appearance of pairs of complementing alleles, can also be generated by haplo-specific lethals.

The complex complementation pattern of BELYAEVA *et al.* (1980) is of special interest, since it is associated with haplo-specific lethal mutations. Four mutations included in the pattern are haplo-specific lethals, as well as three others that are included in two simple complementation groups located nearby. However, in this case, the complementation pattern would not be substantially altered by exclusion of the haplo-specific lethal mutations. The presence of haplo-specific lethals in this pattern implies the probable inclusion of hypomorphs within the sample of fully lethal alleles. It is possible that these mutations generate the complex complementation behavior, although there is no definitive model available to explain why.

The final artifact associated with haplo-specific autosomal lethals is a specific case but possibly an important one. If no fully lethal allele of a locus exists, then each cryptic haplo-specific allele isolated may be allocated to a separate complementation group. The absence of a fully lethal allele may occur by chance or, conceivably, as a result of a special genetic circumstance.

As was noted in the introduction to this article, there are several descriptions of instances of haplo-specific lethals (or extreme semilethals) in the literature. However, it is a little puzzling that the isolation of haplo-specific lethal mutations is rarely acknowledged in "saturation" studies of recessive lethals, given the frequency with which they were found both in this study and in the work of BELYAEVA *et al.* (1980). In *X* chromosome studies, in which haplo-specific lethals are generally isolated without extraneous lethals, they would most probably be rejected; the quantification of semilethality in a complementation test is tiresome. Even in the in-depth study of JUDD, SHEN and KAUFMAN (1972), the only semilethals retained exhibited visible phenotypes; of the ten described among 121 mutations, two (tko^{25t} and $l(1)zw2^{a3}$) were haplo-specific lethals, and eight others might have appeared so in small samples.

In autosomal studies, identifiable (as opposed to disguised) haplo-specific lethals are probably rejected as false positives when homozygous segregants appear in ostensibly balanced lethal stocks—once again, a seemingly legitimate simplification. Unfortunately, this may have resulted in the loss of useful information since knowledge of the existence of haplo-specific lethals at a locus is perhaps the best signal that problems might be encountered as a result of the artifact described. Other means of handling possible instances of spurious complementation due to haplo-specific lethal mutations are extremely arduous,

requiring either the demonstration that no extraneous lethals exist or their removal by recombination.

CONCLUSIONS

Haplo-specific lethals form a class of mutations that would be expected to occur at any locus at which more than a trace level of activity is required for completion of development providing the locus is not dosage compensated in segmental haploids. There is no reason to suppose that haplo-specific lethal mutations indicate any special mechanism; they are explicable as one manifestation of hypomorphy, equivalent to, but distinct from, semilethality.

The importance of haplo-specific lethal mutations is not intrinsic but methodological. When complementation tests are carried out with autosomes, in particular, it is possible unknowingly to include haplo-specific lethal mutations within a sample of fully lethal mutations. Complementation tests including disguised haplo-specific lethal mutations will frequently, but spuriously, suggest complementation between allelic mutations. The existence of haplo-specific lethal mutations would be expected to account for at least some of the instances of interallelic complementation described in the literature. This applies both to instances that might be ascribed to complex loci and to those in which the conventional explanation would involve the interaction of protein subunits. In the extreme case, in which no fully lethal alleles are available, the artifact can lead to the description of alleles of the same gene as nonallelic, thereby incorrectly suggesting the presence of additional essential genes.

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