HETEROGENEITY OF LETHALS IN A "SIMPLE" LETHAL COMPLEMENTATION GROUP

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ABSTRACT

Of 24 ethyl methanesulphonate-induced, recessive-lethal mutations in the region 9E1-9F13 of the X chromosome of *Drosophila melanogaster*, eight fall into a typically homogeneous lethal complementation group associated with the *raspberry* (*ras*) locus. Mutations in this group have previously been shown to be pleiotropic, affecting not only *ras* but also two other genetic entities, *gual* and *pur1*, which yield auxotrophic mutations.—The eight new mutations have been characterized phenotypically in double heterozygotes with *gual*, *pur1* and *ras* mutations. Despite their homogeneity in lethal complementation tests, the mutations prove quite diverse. For example, two mutations have little or no effect on eye color in double heterozygotes with *ras*². The differences between the lethals are allele-specific and cannot be explained as a trivial outcome of a hypomorphic series.—Taken alone, the lethal complementation studies mask the complexity of the locus and the diversity of its recessive lethal alleles. By extension, we argue that the general use of lethal saturation studies provides an unduly simplified image of genetic organization. We suggest that the reason why recessive lethal mutations rarely present complex complementation patterns is that complex loci tend to produce mutations that affect several subfunctions.

THE great majority of recessive lethal mutations fall into simple cistrons (for a discussion of this phenomenon, and some of the exceptions, see NASH and JANCA 1983). Taken at face value, this observation would seem to indicate that the essential functions defined by recessive lethals are encoded within simply organized genes. In contrast, the elaborate patterns of complementation associated with many loci that yield "visible" mutations suggest that nonessential functions are encoded in much more complex genetic structures. It is evident that this distinction is spurious, for some loci that yield complex complementation patterns with visible mutants also yield recessive lethal alleles.

NASH *et al.* (1981) reported that *raspberry-lethal* (*ras-l*) mutations in *D. melanogaster* fail to complement with all of three mutually complementary classes of recessive, nonlethal mutations (two kinds of purine nucleoside auxotrophs and *ras*, which is defective in pteridine synthesis). A genetic complex associated with purine metabolism appears to be defined by this phenomenon, and the

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most direct interpretation of the results is that *ras-l* mutations are multifunctional defects.

In the present report we show that *ras-l* mutations form a single lethal complementation group. However, their pattern of complementation with the nonlethal mutations in the complex is heterogeneous. These results lead us to conclude that the power of lethal complementation tests to reveal genetic complexity is seriously restricted.

MATERIALS AND METHODS

Culture methods: The culture methods used are described fully by EL KOUNI and NASH (1974). Cultures were normally grown on a yeast-sucrose medium except that, when nutritional studies were involved, modified SANG's (1956) medium was used. Experiments carried out on SANG's medium (and their yeast-sucrose controls) used axenic culture. Unless otherwise stated, flies were reared at 25°.

Stocks: Unless otherwise noted, descriptions of genetic variants used are found in LINDSLEY and GRELL (1968).

Production and cytogenetic localization of mutations in the 9E1-9F13 region: The 24 mutations used were isolated after EMS treatment of adult *y cv v f* males, as described by NASH and JANCA (1983). As an integral part of the mutation screen, which is shown in Figure 1, the mutations were localized cytologically by crosses involving *Df(1)217*, *y cv ras⁻ v f*; *Df(1)59*, *y cv ras⁻ v f* (Figure 2); *Df(1)203*, *y cv ras⁻ v f* (NASH and JANCA 1983) and *Df(1)v64f* (LEFEVRE 1969). The breakpoints of these deficiencies are given in Table 1.

Each new mutation was maintained in two stocks; in one it was balanced against the *FM6* chromosome and in the other it was maintained in males, covered by the transposition, *Dp(1;2)v⁺63i* (LEFEVRE 1969), against the attached-X chromosome *C(1)DX*, *y w f*.

Eight of these mutations have been called "haplo-specific lethals" by NASH and JANCA (1983) because they are fully lethal only when uncovered by a deficiency. For purposes of simplification all will be referred to as lethals in this report, except where a distinction is essential.

Lethal complementation tests: All possible pairwise crosses (including reciprocals) were performed with 22 of the 24 mutations. These crosses used *y cv l₁ v f/FM6* females and *y cv l₂ v f/Y*; *Dp(1;2)v⁺63i/+* males as parents, where *l₁* and *l₂* are two independently isolated recessive lethals. Among the progeny, the ratio of *y cv v f* (*Dp*-free) and *y cv f* (*Dp*-bearing) females was taken as an indication of complementation behavior. The two mutations not included in the complete test were tested against a representative panel of alleles. These were sufficient to give unequivocal assignments to known complementation groups. The tests were not completed because of stock breakdowns.

Background substitution of *ras-l* mutations: In order to distinguish allele-specific differences from the effects of genetic background, seven of the eight members of the complementation group that includes *rasberry-lethals* were passed through the crossing scheme outlined in Figure 3. The final substitution of the proximal portion of the X chromosome was not accomplished (after three attempts) for the eighth mutation. The half-substituted stock was included in subsequent tests.

This scheme does not guarantee equivalent substitution of the genome for all strains. It achieves an effective randomization of the autosomes as a result of repeated outcrossing; a large proportion of the X chromosome is substituted quasi-mechanically.

Complementation tests with nonlethal mutations in the *gual-pur1-ras* complex: The phenotypes associated with the eight *ras-l* alleles in the sample (in both "backgrounds") were assessed in double heterozygotes with *pur1¹*, *pur1²*, *gual^{1a}* (FALK and NASH 1974a; JOHNSON, WOLOSHYN and NASH 1979) and *ras²*. The crosses employed

FIGURE 1.—Protocol employed for isolation of recessive lethals and their cytogenetic localization. “*Dp*” refers to *Dp(1;2)u⁺63i*.

were similar to those described by NASH *et al.* (1981). Details are included in the legends to Tables 3–5.

Characterization of the pteridine content: Pteridines were extracted by a modification of the method of FAN *et al.* (1976). Samples of adult females of appropriate genotype (*ras-l/ras²*) were collected both within 12 hr of eclosion and 3–4 days after eclosion. They were thoroughly homogenized on ice with a freshly made 1:1 mixture of 10% ammonia and chloroform in a 15 ml Dounce homogenizer with a B pestle. In general, 200 flies were homogenized in 1 ml of liquid; when fewer flies were available, the volume of liquid was reduced proportionately. The homogenate was centrifuged for 15 min at 12,000 rpm in a microcentrifuge. An equal volume of 10% aqueous trichloroacetic acid was mixed with the aqueous supernatant (the chloroform being trapped under the sedimented debris), and the resulting precipitate was removed by

FIGURE 2.—a, The study region in a wild-type female. Presumed locations of all 13 bands identified by ZHIMULEV, SEMESHIN and BELYAEVA (1981) are shown. Those bands indicated with broken lines are not evident in the photograph. b, *Df(1)217/+*, unsynapsed. When synapsed, all chromomeric material distal to 9F1-2 in the deficiency chromosome appears to pair distal to 9A. The bands labeled 9F1-2 usually synapse, despite the appearance of qualitative difference in this photograph. c, *Df(1)59/+*, unsynapsed. d, Region d' (from 2c) including *Df(1)59*. e, Region e' (from 2c) showing the wild-type homolog. When synapsed, *Df(1)59* shows clear evidence that a small portion of 9A is juxtaposed to 10A. If well squashed, this material appears separated from 10A by a narrow interband region, which is most reasonably interpreted as material immediately distal to 9F12. Scale bars represent 10 μ m.

centrifugation. Then, 80 μ l of the supernatant was spotted, in consecutive 8 μ l aliquots, on Whatman No. 3 chromatographic paper. After equilibration for 10–16 hr over 10% ammonia, the chromatographs were developed for 10 hr by descending chromatography with 1-propanol:1% ammonia (1:1) over 10% ammonia. The four extracts from a

FIGURE 3.—Protocol used in generating $lz\ ras-l\ m$ stocks. “ Dp ” refers to $Dp(1;2)v^*63i$. The three possible genotypes (a, b, c) of generation 3 and generation 6 male parents are phenotypically indistinguishable; this is resolved in subsequent generations.

given $ras-l/ras^2$ combination (both ages of flies, both genetic backgrounds) were obtained simultaneously and were run on the same paper together with comparable extracts of ras^+ and ras^2 flies. All flies involved were homozygous for v .

Visual comparisons of chromatographs were made, without quantitation, using an

ultraviolet lamp. Records of chromatographs were made with black-and-white polaroid photography.

Nomenclature: A number of the nomenclatural modifications that will be forthcoming in the revised version of *Genetic Variations of Drosophila melanogaster* (D. L. LINDSLEY, personal communication) have been used here. These include $Dp(1;2)v^{+63i} = Dp(1;2)v^{+63i}$, $Df(1)v^{-64f29} = Df(1)v64f$, $pur1-1 = pur1^1$, $pur1-2 = pur1^2$, and $gual-1^{1a} = gual^1$. In addition, $l(1)EM32$ will become $l(1)9Ea^1$, the group A (*ras*) lethals will remain *ras-l* ($= l(1)9Eb$), group C will become $l(1)9Ec$, group B (including $l(1)EM31$) will become $l(1)9Ed$ alleles and group G will become $l(1)9Ee$.

RESULTS

Cytogenetic localization

Table 1 shows the complementation pattern of the lethals with the four deficiencies. The deficiencies divide the region from 9E1-9F13 into four subregions and the lethals into four groups. The four patterns of complementation are compatible with the cytological observations, with $Df(1)217$, $Df(1)59$ and $Df(1)203$ extending from the left-hand end of $Dp(1;2)v^{+63i}$ (at 9E1) progressively further into the region, and with $Df(1)v64f$ starting to the right of 9E4 and extending into segment 10.

The equivocal situation at the left-hand end of $Df(1)203$, where material of doubtful origin borders the break (see NASH and JANCA 1983), is slightly clarified by the data. No mutation was obtained that is covered by $Dp(1;2)v^{+63i}$ and exposed by either $Df(1)217$ or $Df(1)59$, but not by $Df(1)203$. Therefore, $Df(1)203$ removes or inactivates all material at the left-hand end of $Dp(1;2)v^{+63i}$ that yielded mutations in this study. The origin of the extra material remains problematical.

There were six cases, noted in Table 1, in which "escapers" arose in crosses that, nevertheless, appear to indicate that the mutant is exposed by a deficiency. In each of these cases, there was a severe reduction in the number of experimental flies relative to controls (minimum $\chi^2_{(1)} = 10.7$, $P < 0.005$). In three cases (A17, D12 and Q21) the mutation behaved as a full lethal in all other tests, and the single escaper could even have resulted from misclassification. In the other three cases of escapers (B4, F16 and M14) the "lethal" was, in fact, a haplo-specific lethal, and the deficiency ($Df(1)v64f$) was introduced from the male parent (see Figure 1). In two cases (F16 and M14), escapers were sufficiently common to suggest a real effect. NASH and JANCA (1983) point out the possibility that these escapers result from a maternal effect analogous to that discovered by ROBBINS (1983).

In no case where escapers were found does it seem reasonable to contend that the mutation is not exposed by the deficiency concerned; in five cases this would result in a complementation pattern incompatible with the remaining genetic and cytogenetic data. The sixth case (A17) turns out to have four alleles, all of which unambiguously exhibit the same complementation pattern.

The complementation tests between the induced lethals

Table 2 summarizes the results of the lethal complementation tests between 22 of the 24 mutations. The mutations have been grouped to reveal the

TABLE 1
Complementation tests of lethals in the region 9E1-9F13 with four deficiencies

Lethal Mutation	<i>Df(1)217</i> (9A; 9E7-8)	<i>Df(1)59</i> (9A; 9F10-11)	<i>Df(1)203</i> (9E1-2; 9F13)	<i>Df(1)v64f</i> (9E7-8; 10A1-2)	Region defined
B13	—	—	—	35/55 (+)	9E1-2; 9E4
D12	—	1/12 (—)	—	+	
E20	—	—	—	+	
F1	—	—	—	+	
F19	—	—	—	+	
G4	—	—	—	+	
H6	—	—	—	+	
H25	—	—	—	+	
<i>J5</i>	—	—	—	+	
K27	—	—	—	+	
N23	—	—	—	+	
A17	—	—	—	1/40 (—)	9E7-8
<i>G16</i>	—	—	—	—	
H22	—	—	—	—	
<i>M27</i>	—	—	—	—	
<i>Q10</i>	—	—	—	—	
Q21	1/109 (—)	—	—	—	
<i>F16</i>	+	—	—	15/38 (—)	9F1-2; 9F10-11
G19	+	—	—	—	
H21	+	—	—	—	
<i>A15</i>	+	+	—	—	9F12; 9F13
<i>B4</i>	+	+	—	1/26 (—)	
F21	+	+	—	—	
<i>M14</i>	+	+	—	7/34 (—)	

The crosses used for the tests are shown in Figure 1. Haplo-specific lethal mutations (see NASH and JANCA 1983) are indicated in italics. Numerical data are shown where *Df*/mutation double heterozygotes did survive, although significantly less well than did controls. Cases where these were presumed to be "escapers" (—) are discussed in the text. The one case where complementation is presumed to have occurred (+) shows only a marginal effect ($\chi^2_{(1)} = 4.1$ $0.05 > P < 0.01$); the deviation may therefore have arisen by sampling error.

complementation pattern: There are eight complementation groups (A–H), with 8, 4, 3, 3, 1, 1, 1 and 1 members, respectively.

For simplicity, the data in Table 2 have been reduced as far as possible: Crosses yielding zero experimental progeny (*Dp(1;2)v⁺63i*-free females) are indicated as "—." Usually, crosses showing no significant reduction from experimental:control ratio of unity (at the 5% level) are shown as "+." There are three exceptions to this rule, all in complementation group B. G16 and M27 homozygotes and a G16/M27 heteroallelic combination are assigned their position in the matrix (and indicated as "—") because of their behavior as haplo-specific lethals, as explained by NASH and JANCA (1983). The justification is based on their severely reduced viability in heteroallelic combinations with A17 and H22, which are fully lethal mutations.

TABLE 2

Complementation matrix between 22 recessive lethal mutations in the region 9E1-9F13

Female parent (mutation/ <i>FM6</i>)	Male parent (mutation/											
	D12	F1	F19	H6	H25	K27	N23	<i>J5</i>	A17	H22	<i>G16</i>	<i>M27</i>
D12	—	—	—	—	—	—	—	—	+	+	+	+
F1	—	—	—	—	—	—	—	(—) 15/48	+	+	+	+
F19	—	—	—	—	—	—	—	(—) 5/41	+	+	+	(+) 11/23
H6	—	—	—	—	—	—	—	—	+ ^a	+	+	+
H25	—	—	—	—	—	—	—	—	+	+	+	+
K27	—	—	—	—	—	—	—	—	+	(+) 30/48	+	+
N23	—	—	—	—	—	—	—	—	+	+	+ ^a	+
<i>J5</i>	—	(—) 8/38	(—) 10/27	—	—	(—) 1/29	—	(—) 13/33	+	+	+	+
A17	+	+	+	+	+	+	+	+	—	—	(—) 11/38	(—) 5/34
H22	+	+	+	+	+	+	+	+	—	—	—	(—) 1/18
<i>G16</i>	+	+	+	+	+	+	+	+	—	—	(—) 24/38	(—) 32/33
<i>M27</i>	+	+	+	+	+	+	+	+	—	—	(—) 21/37	(—) 34/34
B13	+	+	+	+	+	+	+	+	+ ^a	+ ^a	+	+
E20	+	+	+	+	+	+	+	+	+ ^a	(+) 28/47	+ ^a	+
G4	+	+	+ ^a	+ ^a	+	+ ^a	+	+	+	+ ^a	+ ^a	+
A15	+	+	+	+	+	+	+	+	+	+	+	+
B4	+	+	+	+	+	+	+	+	+	(+) 53/77	+	+
M14	+	+	+	+	+	+	+	+	+	+	+	+
F21	+ ^a	+	+	+	+	+	+	+	+	+	+	+
H21	+	+	+	+	+	+	+	+	+	+	+	+
Q21	+	+	+	+	+	+	+	+	+	+ ^a	+	+
F16	+	+	+	+	+	+	+	+	+	+	+	+
Group	A						B					

Cases of allelism (lack of complementation) are indicated as “—,” complementation is indicated heterozygotes (or homozygotes, where the mutation is the same in each parent) lacking and and is indicated parenthetically in the table. Haplo-specific lethals are indicated in italics. The mu- indicated in the bottom row.

^a Indicates instances where the number *Dp(1;2)u⁺63i*-free females significantly exceeds the con-

*Y; Dp(1;2)v*63i/+)*

B13	E20	G4	A15	B4	M14	F21	H21	Q21	F16
+	+	+	(+) 32/56	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	(+) 37/56	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	(+) 33/56	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
-	-	-	+	+	+	+	+	+	+
-	-	-	+	+	+	+	+	+	(+) 33/54
-	-	-	+	+	+	+	+	+	+
+	+	+	(-) 9/28	(-) 3/27	(-) 26/54	+	+	+	+
+	+	(+) 51/73	(-) 4/55	(-) 1/60	(-) 2/24	+	+	+	+
+	+	+	(-) 14/93	(-) 4/48	(-) 12/49	+	+	+	+
+	+	+	+	+	+	-	+	+	+
+	+	+	+	+	+	+	-	+	+
+	+	+	+	+	+	+	+	-	+
+	+	+	+	+	+	+	+	+	(-) 14/67
C			D			E	F	G	H

as "+." Data are given when the result is ambiguous; they represent the numbers of double carrying *Dp(1;2)v*63i*, respectively. The resolution of these ambiguities is discussed in the text tations have been ordered in such a way as to reveal the eight complementation groups (A-H)

trol number.

With these three exceptions, the numerical data are included only when experimental females do survive, although significantly less well than do controls. Sixteen cases involve heteroallelic combinations where one or both alleles are haplo-specific lethal mutations. NASH and JANCA (1983) have argued that these cases represent allelism. Five cases are homozygous haplo-specific lethals.

The remaining nine numerical entries are presumed to be instances of complementation. These nine cases, while individually significant ($3.8 < \chi^2_{(1)} < 6.5$, $0.05 < P < 0.01$), are not significant when taken as a whole: There are 382 entries in the matrix that seem to represent complementation, and of these 5% (or 19.1) would be expected to give $\chi^2_{(1)}$ -values greater than 3.8, half of them as a result of reduced survival of experimental flies. Thus, the number of deviants of this kind is at the minimum expected. Increasing sample sizes would not reduce this number, but might increase it by revealing more subtle viability differences. The attribution of these cases to sampling error is compatible with the observation that none are confirmed in the reciprocal cross.

An equivalent number of cases in which significantly more experimental flies than control flies survive would be expected by chance. In fact, more than twice as many cases (21) were found; these are indicated by asterisks. In all probability, therefore, some of these are not chance events. The most likely explanation is lowered viability of control individuals. A large proportion of these cases have one parent in complementation group C; the biological significance, if any, of this clustering is not evident.

Mutations G19 and Q10 were not included in the complete matrix because one of the parental stocks involved was lost before the matrix was completed. Both were tested reciprocally against a panel of representative alleles from all complementation groups. All results obtained from these mutations were compatible with allocation of G19 to complementation group F and of Q10 to group B. It was felt that reconstruction of the missing stocks would not provide useful additional information.

In total, the complementation tests, including the tests carried out with haplo-specific lethals, generate an exceedingly conventional complementation pattern. The eight complementation groups are exclusive of each other, and there are no examples of complex loci. The survival of heteroallelic combinations in complementation groups A, B and D can be explained as a result of haplo-specific lethal mutations, not of *bona fide* interallelic complementation (NASH and JANCA 1983). The members of a given complementation group fall into a single cytological region: A and C are in the region 9E1-2 to 9E4; B and G are in 9E7-8; E and H are in 9F1-2 to 9F10-11; and D and F are in 9F12-9F13.

Complementation tests between the members of complementation group A and nonlethal mutations in the *gual-purl-ras* complex

The *gual-purl-ras* complex is in region 9E1-9E4 (JOHNSON, WOLOSHYN and NASH 1979), probably at 9E3-9E4 (ZHIMULEV, SEMESHIN and BELYAEVA 1981); this co-localization with complementation groups A and C suggests that members of either complementation group A or C are *ras-l's*. J5, the haplo-specific

lethal member of complementation group A, gives homozygous and hemizygous flies which are clearly *ras* in eye color, suggesting that the remaining members of this complementation group are *ras-l*'s. All 24 mutations were tested in double heterozygotes with *ras*² in a *v* background to facilitate observation of the *ras* phenotype. Six members of group A (including J5) gave *ras*-eyed flies and are therefore *ras-l*'s; none of the remaining 18 mutants were obviously different in phenotype from *v/v*.

The two group A mutations, F1 and F19, gave *ras*⁺ eye-color in double heterozygotes with *ras*², so they were not classified as *ras-l* on the basis of this test. The allelic variation implied by this observation was further investigated.

In order to differentiate possible allelic variation from the effects of modifiers and of experimental fluctuations, each group A mutation was placed in a different genetic background, as described in MATERIALS AND METHODS. The original and the substituted stocks were then used to investigate the phenotype of each mutant in heteroallelic combination with *gual*^{ts}, *pur1*¹, *pur1*² and *ras*². In the cases of the auxotrophic mutations, survival of the heterozygotes on axenic yeast-sucrose and SANG's nucleoside-free media was examined at 25° and 29°. The *ras*² combinations were examined chromatographically for pteridine content. Sample alleles from the remaining seven complementation groups gave no evidence of allelism with any of the four nonlethals (results not shown).

Survival of *gual*^{ts}/*ras-l* combinations: *gual*^{ts} homozygotes and hemizygotes show about 5% survival at 29° and 50% survival at 25° when grown on nucleoside-free defined medium (FALK 1973). An approximately similar temperature-dependent differential is found when this mutant is exposed by a deficiency (JOHNSON, WOLOSHYN and NASH 1979). The same genotypes survive as well as controls on yeast-sucrose medium at either temperature. Table 3 shows the survival of the new *ras-l* mutations in combination with *gual*^{ts}.

On yeast-sucrose medium the *gual*^{ts}/*ras-l* flies show high levels of survival in all cases. In only one case (*lz* F1 *m*) is survival significantly, although marginally, depressed.

On defined medium, all 32 crosses demonstrate significant depression of survival. Seven mutations (D12, F1, F19, H6, H25, K27 and N23) show low survival (less than 6%) at 29° and seem to be at least as extreme as *gual*^{ts} itself. The eighth mutation (J5) survives considerably better and would therefore appear to be less defective than *gual*^{ts}. The J5 result is reflected in the results at 25°. At 25°, K27 also survives relatively well in both backgrounds; the remainder all show severely depressed survival compared with *gual*^{ts}/*Df* (JOHNSON, WOLOSHYN and NASH 1979). This latter phenomenon, in which the heteroallelic combination has a more extreme phenotype than the deficiency heterozygote, was previously described by NASH *et al.* (1981).

Survival of *pur1*¹/*ras-l* combinations: *pur1*¹ is a mutation that shows less than 5% survival on nucleoside-free medium (FALK and NASH 1974a); its supplementation and complementation behavior suggest a main defect in *pur1* function and a minor defect in *gual* function (FALK 1973). Deficiency heterozygotes survive poorly on yeast-sucrose medium (JOHNSON, WOLOSHYN and

TABLE 3

Survival of *ras-l/gua1^u* combinations under a variety of growth conditions

Lethal mutation	Background	Medium			
		Yeast-sucrose		Sang's	
		25°	29°	25°	29°
D12	<i>y cv f</i>	385/352	364/326	* {	6/437*
	<i>lz m</i>	227/211	140/152		5/114*
F1	<i>y cv f</i>	129/118	234/267		10/162*
	<i>lz m</i>	112/103	127/175*		13/140*
F19	<i>y cv f</i>	93/103	176/202		2/129*
	<i>lz m</i>	267/254	191/211		5/162*
H6	<i>y cv f</i>	195/196	207/203	* {	10/275*
	<i>lz m</i>	541/534	409/401		61/587*
H25	<i>y cv f</i>	189/167	138/166		15/127*
	<i>lz m</i>	243/240	337/329		19/171*
J5	<i>y cv f</i>	218/189	84/78	* {	98/183*
	<i>lz m</i>	497/533	413/428		248/303*
K27	<i>y cv f</i>	174/189	241/242	* {	130/196*
	<i>lz f</i>	468/479	409/360		33/124*
N23	<i>y cv f</i>	157/150	167/144		4/182*
	<i>lz m</i>	289/325	152/175		5/221*

Data show the number of *ras-l/gua1^u* flies/number of *FM6/gua1^u* flies derived from the cross *ras-l/FM6* × *gua1^u/Y*. Complete substitution of K27 background was not accomplished (see text). Haplo-specific lethal is indicated in italics.

* Indicates χ^2 values ($P < 0.05$): (1) in cases where the segregation represents a deviation from the expected 1:1 ratio, the asterisk follows the data; (2) in cases where the two segregations for a given lethal differ (by contingency χ^2), the asterisk precedes the data.

NASH 1979). Table 4 shows the survival of the new *ras-l* mutations in combination with *pur1¹*.

Previously reported results with *ras-l* mutations (NASH *et al.* 1981) indicated that *pur1¹/ras-l* exhibits similar behavior to *pur1¹/Df* combinations, although to a less extreme degree. They also reported that, at 25°, the combination with an EMS-induced *ras-l* was substantially more viable than with two X-ray-induced mutations. In this respect, the behavior of only one (H6) of the novel batch of mutations is as extreme as the EMS-induced lethal tested previously. K27, in combination with *pur1¹*, survives poorly at 29°, but does well at 25°, on yeast-sucrose medium. It is not possible to ascribe the somewhat reduced survival of F1 (in *y cv f* background) and J5 (in *lz m* background) to allelic characteristics, because in each case, survival was normal in the alternative background.

Except for confirming that all eight lethals are allelic with *pur1¹*, the results on defined medium are relatively uninformative. Survival rate falls into the comparatively narrow range of 0–11%; 30 of the 32 results are below 5%. Two mutations (F19 and H25) showed survivors in all four tests, and one (H6)

TABLE 4

Survival of *ras-l/pur1*¹ combinations under a variety of growth conditions

Lethal mutation	Background	Medium			
		Yeast-sucrose		Sang's	
		25°	29°	25°	29°
D12	<i>y cv f</i>	447/434	* { 400/461*	10/550*	0/358*
	<i>lz m</i>	166/178	{ 148/123	2/239*	0/169*
F1	<i>y cv f</i>	109/120	* { 90/174*	3/211*	0/165*
	<i>lz m</i>	92/91	{ 80/87	9/200*	2/250*
F19	<i>y cv f</i>	402/393	462/467	5/380*	8/276*
	<i>lz m</i>	146/138	188/204	2/199*	4/157*
H6	<i>y cv f</i>	* { 101/313*	2/265*	0/323*	0/190*
	<i>lz m</i>	{ 16/155*	5/295*	0/181*	0/347*
H25	<i>y cv f</i>	* { 128/104	150/129	* { 7/103*	2/46*
	<i>lz m</i>	{ 283/324	242/225	{ 4/236*	1/244*
<i>J5</i>	<i>y cv f</i>	127/154	73/69	0/56*	0/154*
	<i>lz m</i>	292/267	108/151*	7/188*	0/183*
K27	<i>y cv f</i>	116/111	* { 19/97*	3/91*	0/58*
	<i>lz f</i>	564/565	{ 156/467*	4/114*	2/56*
N23	<i>y cv f</i>	295/282	193/151*	* { 47/436*	2/253*
	<i>lz m</i>	383/406	190/194	{ 4/402*	0/230*

Data show the number of *ras-l/pur1*¹ flies/number of *FM6/pur1*¹ flies derived from the cross *ras-l/FM6* × *pur1*¹/*Y*. Complete substitution of K27 background was not accomplished (see text). Haplo-specific lethal is indicated in italics.

* Indicates χ^2 values ($P < 0.05$): (1) in cases where the segregation represents a deviation from the expected 1:1 ratio, the asterisk follows the data; (2) in cases where the two segregations for a given lethal differ (by contingency χ^2), the asterisk precedes the data.

showed survivors in none; in general, however, there was little clear-cut correlation between the four results for a given mutation, so that the limited variation cannot be ascribed to allele-specific differences.

Survival of *pur1*²/*ras-l* combinations: The *pur1*² allele is similar to *pur1*¹, except that there is no evidence of a subsidiary effect on the *gual* function. *pur1*²/*Df* double heterozygotes show low viability on defined medium and at 29° on yeast-sucrose medium, but survive reasonably well at 25° on yeast-sucrose medium (JOHNSON, WOLOSHYN and NASH 1979). Among the sample of *ras-l*'s tested by NASH *et al.* (1981), the X-ray-induced mutations behaved rather like deficiencies, but the EMS-induced mutation was less extreme. Unlike *pur1*¹/*ras-l*, *pur1*²/*ras-l* flies survive on defined medium supplemented with adenosine and guanosine; indeed, they appear to be resistant to adenosine toxicity (NASH *et al.* 1981).

Table 5 shows the results of our present experiments using *pur1*². At 25° on yeast-sucrose medium, only *lz* N23 *m* exhibits lowered viability. At 29°, *J5* and K27 have significantly reduced viability in both genetic backgrounds. In the case of K27, the result appears to correspond with that found with *pur1*¹.

TABLE 5
Survival of *ras-l/pur1*² combinations under a variety of growth conditions

Lethal mutation	Background	Medium			
		Yeast-sucrose		Sang's	
		25°	29°	25°	29°
D12	<i>y cv f</i>	680/718	488/534	* { 18/664*	* { 0/219*
	<i>lz m</i>	138/134	307/345	{ 16/215*	{ 6/138*
F1	<i>y cv f</i>	* { 186/141*	166/147	* { 38/112*	* { 84/173*
	<i>lz m</i>	{ 128/160	180/160	{ 29/147*	{ 22/95*
F19	<i>y cv f</i>	563/571	439/396	46/285*	12/225*
	<i>lz m</i>	394/387	336/362	55/262*	22/308*
H6	<i>y cv f</i>	271/228	160/237*	1/259*	0/188*
	<i>lz m</i>	232/244	81/104	1/305*	1/299*
H25	<i>y cv f</i>	56/61	35/25	* { 52/102*	* { 25/33
	<i>lz m</i>	193/188	261/211*	{ 50/271*	{ 18/74*
J5	<i>y cv f</i>	80/88	68/108*	0/76*	0/124*
	<i>lz m</i>	186/219	202/253*	1/242*	0/141*
K27	<i>y cv f</i>	298/316	126/190*	0/214*	0/96*
	<i>lz f</i>	655/657	446/536*	0/178*	0/70*
N23	<i>y cv f</i>	* { 315/289	240/212	* { 40/362*	* { 7/82*
	<i>lz m</i>	{ 278/382*	171/196	{ 72/384*	{ 101/283*

Data show the number of *ras-l/pur1*² flies/number of *FM6/pur1*² flies derived from the cross *ras-l/FM6* × *pur1*²/*Y*. Complete substitution of K27 background was not accomplished (see text). Haplo-specific lethal is indicated in italics.

* Indicates χ^2 values ($P < 0.05$): (1) in cases where the segregation represents a deviation from the expected 1:1 ratio, the asterisk follows the data; (2) in cases where the two segregations for a given lethal differ (by contingency χ^2), the asterisk precedes the data.

The result with J5 is unexpected, because it is the weakest mutant with *gual*^{ts} and is only semilethal. H6 shows overall survival of 70% (which is significantly low) in combination with *pur1*², although in the *lz m* background, survival, while reduced, is not significantly low, based on a smaller than average sample size. However, in general, the viability of the new lethals in heteroallelic combinations with *pur1*², on yeast-sucrose medium, was higher than that described by NASH *et al.* (1981).

On SANG's (1956) medium, all mutations are clearly allelic with *pur1*². The somewhat reduced viability of H6, J5 and K27 with *pur1*² on yeast-sucrose medium is reflected by extremely low survival (less than 0.5%) on SANG's medium. *pur1*²/D12 is consistently of fairly low viability (between 0 and 7.4%). The remaining four mutations (F1, F19, H25 and N23) range, in various tests, between 5 and 75% viability. For these mutations, the data are massively inconsistent, with variation failing to partition consistently between mutations, backgrounds or growth temperatures.

The viability of *pur1*²/*ras-l* on nucleoside-free medium is extremely variable; nevertheless, a great deal of the variability is allele-specific. The relatively weak

TABLE 6

Estimates of relative drosoppterin content of *ras-l/ras²* based on chromatographic observations

Lethal mutation	Younger flies (<12 hr)		Older flies (3- to 4-days old)	
	<i>y cv f</i>	<i>lz m</i>	<i>y cv f</i>	<i>lz m</i>
D12	3	2	3	2
F1	8	8	9	9
F19	7	7	9	8
H6	3	3	1	1
H25	3	2	2	1
<i>J5</i>	5	3	5	4
K27	3	3 ^a	3	3 ^a
N23	3	1	4	5

Numbers represent subjective estimates on a scale of 1-9, where 2 represents the intensity of drosoppterin fluorescence in *ras²* homozygotes, and 8 represents the intensity in wild type, for flies of similar age. Haplo-specific lethal is indicated in italics.

^a K27 substitution incomplete; a *lz K27 v f* chromosome was used.

and inconsistent effects of temperature are somewhat surprising, given that survival of *pur1²/Df* is strongly dependent on temperature (JOHNSON, WOLOSZYN and NASH 1979).

Eye color and pteridine content in *ras²/ras-l* combinations

Visual comparison between eye color of individual flies is difficult because pteridine content of both wild-type and, to a much greater extent, *ras*-eyed flies appears to change rapidly in the first few days after eclosion. In consequence, pteridines were extracted from bulk samples of *ras²/ras-l* flies and were separated by chromatography. For each lethal mutation, in both backgrounds, extracts of flies less than 12 hr old and of 3- to 4-day-old flies were prepared on the same day. These were run chromatographically with comparable extracts of *ras⁺/ras⁺* and *ras²/ras²*. The chromatographs were analyzed subjectively under ultra-violet illumination and from black-and-white polaroid photographs. A number of minor, mainly quantitative variations were found in a variety of pteridines, but these have been ignored, in favor of a categorization of drosoppterin contents relative to *ras⁺/ras⁺* and *ras²/ras²*. The results are reported in Table 6. On the numerical scale used (1-9), *ras⁺/ras⁺* is arbitrarily given a value of 8 and *ras²/ras²* a value of 2.

We have studied the actual eye color of flies to check the fidelity of our chromatographic results; the two measures are congruent. Flies with a high drosoppterin content (8, 9) are indistinguishable from *ras⁺*, while flies with low drosoppterin content (1-3) are like *ras²* individuals. We do not have sufficient confidence in the objectivity of our comparisons of whole organisms to suggest that there is an exact equivalence between them and the chromatographic observations. However, in three instances, subtle differences in the chromato-

graphic pattern are reflected in whole flies: First, the eyes of young N23 flies are much like *ras*², but pigmentation intensifies to a greater extent with age; we have not, however, identified the differences between the two N23 stocks implied by the chromatographic results. Second, the difference between *y cv J5 f* and *lz J5 m* is largely explained by a polymorphism in the *y cv J5 f* stock; a small proportion of the *ras*²/*J5* flies have intensely pigmented, but still distinguishably *ras*-colored, eyes, although the majority have lighter eyes similar to those of *ras*²/*lz J5 m*. The factor responsible is X-linked, but we have not identified its map position. Finally, the slight reduction in pigmentation in young F19 flies is real, although only evident for a few hours after eclosion, and then only if a most careful comparison is made.

None of our observations of whole flies confirms the existence of phenotypes corresponding to values 1 or 9 in Table 6; it is possible, therefore, that these values may have been necessary simply to record experimental error.

DISCUSSION

The 9E1-9F13 region: Two previous studies (ZHIMULEV *et al.* 1981; LEFEVRE 1981) have been published on the complementation pattern in 9E1-9F13. ZHIMULEV, SEMESHIN and BELYAEVA (1981) also published a definitive electron microscopic analysis of the region, showing a total of 13 bands in the region. This result is represented in Figure 2a.

Although ZHIMULEV *et al.* (1981) discuss the entire study region, they isolated lethal mutations only in its rightmost portion, 9F12-13, where two lethal loci, *l(1)BP1* and *l(1)BP3*, were found. It is possible that these loci correspond to our complementation groups D and G.

LEFEVRE (1981) reports a total of seven lethal complementation groups in the study region, none of them in 9F12 or 9F13. We have not concerned ourselves with equivalences between LEFEVRE's mutations in the right-hand (9F) portion of our study region and our own mutations. LEFEVRE shows four complementation groups in 9F, including *l(1)S12*, which we have reason to believe may reside in region 9E (see below).

In the left-hand portion (9E), we have ordered the complementation groups by recombination, using *lz* and *m* as outside markers. Results will be presented elsewhere. The order is A—C—B—G. A, C and B are very close together and G is some considerable distance to the right. These four groups, which appear to be contained in the region 9E, thus appear not to include all three of the groups reported by LEFEVRE (1981), since he shows one complementation group (EM32) to the left of *ras*, which is our group A.

LEFEVRE has kindly supplied examples of *EM31*, *EM32* and *l(1)S12* mutations, and we have performed preliminary experiments intended to clarify the situation. The *EM32* mutation is allelic with none of the representative panel of mutations that we studied. We assume that it is located to the left of *ras*, as suggested by LEFEVRE (1981), and hence in 9E1-2. The *EM31* mutation which G. LEFEVRE (personal communication) describes as a breakpoint effect of *In(1)9B2-3;9E7-8* is allelic only with an allele of complementation group B. LEFEVRE's *l(1)S12* allele is presumed to be associated with an inversion

9E-F;20A4-5. It is semilethal, and the surviving males are sterile. Our data suggest allelism with only group B mutants.

A range of possibilities therefore exists concerning the region 9E. Our results indicate a minimum of four lethal complementation groups. Two of these groups (A and C) are in 9E3-9E4. LEFEVRE's (1981) *EM32* appears to be another group (presumably located in 9E1-2), which was not represented in our sample. Two other groups, B and G, appear to be located in 9E7-8. (Note that bands 9E5 and 6 were not identified in the electron microscopy studies of ZHIMULEV, SEMESHIN and BELYAeva (1981).) Complementation group B and *EM31* are clearly synonymous. It is probable that *l(1)S12* is also part of this same locus.

At this conventional level of analysis, there is evidence for a minimum of nine (and possibly 11) lethal complementation groups between 9E1 and 9F13, which, together with the *fliK*, *sbr* and *fliG* loci (ZHIMULEV *et al.* 1981), would account reasonably well for the 13 bands found in the region by ZHIMULEV, SEMESHIN and BELYAeva (1981). LEFEVRE's (1981) identification of the region as underpopulated hinges partly on an underestimate of numbers of groups and partly on BRIDGES' (1938) cytological map, which ZHIMULEV, SEMESHIN and BELYAeva (1981) claim inflates the band count by ten.

Heterogeneity of *raspberry-lethal* alleles: In characterizing the *raspberry-lethals*, we had in mind three major questions about their heterogeneity: (1) How great a range of variability exists? (2) Does variability originate from allele-specific differences? (3) Do the three quasi-independent components of *gal-pur1-ras* function vary coordinately?

The data presented attest to the extent of the variability. The most extreme variation occurs with respect to drosopterin production. There is less variation with respect to the *gal* and *pur1* functions. All *ras-l* mutations, in both backgrounds, give evidence for allelism with all three auxotrophic mutations. There are, however, numerous instances where survival differs strongly from the mode.

We approached the question of the origin of this variation by testing each mutation in two stocks, which should have had background differences at least as great as those differentiating the individual *raspberry-lethal* alleles.

Drosopterin content provides the clearest picture with respect to allelic variation. There is no example of radical divergence between the eye color phenotypes exhibited by one mutation in the two backgrounds. Of the two minor differences, J5 is a special case in which a clear-cut polymorphism accrued since the initial mutation was isolated. The second case, N23, if real, involves variation in the rate of pigment formation with age. Most of the large variation in eye pigmentation is, therefore, allele-specific.

When the nutritional phenotypes are examined, the situation is slightly less clear. Table 7 shows an exploratory heterogeneity- χ^2 analysis for 12 blocks of data, each involving one of the three auxotrophic mutations tested under a particular culture regime. The χ^2 is partitioned on the basis of allele-specific differences (A) and on differences depending on "background" (B), leaving a residual or interaction component (R).

TABLE 7
Exploratory χ^2 -analysis of heterogeneity in expression of *ras-l*/auxotroph
double heterozygotes

Auxotrophic mutation	Medium			
	Yeast-sucrose		Sang's	
	25°	29°	25°	29°
<i>gual</i> ^u	T 11.3	24.3	829.9**	424.6**
	A 4.9	13.4	781.1**	393.9**
	B 1.9	0.1	10.1**	2.5
	R 4.4	10.8	38.7**	28.2**
<i>pur1</i> ¹	T 216.0**	632.3**	133.7**	56.0**
	A 194.4**	608.0**	55.9**	40.4**
	B 0.1	11.9**	9.6*	0.7
	R 21.5**	12.4	68.1**	15.0*
<i>pur1</i> ²	T 34.6**	57.9**	397.6**	470.7**
	A 8.1	47.6**	334.6**	405.0**
	B 4.6*	0.1	2.8	0.4
	R 21.9**	11.8	60.1**	65.4**

The analyses were carried out on data shown in Tables 3-5. T, Total heterogeneity of experimental:control ratio, with 15 d.f.; A, heterogeneity between *ras-l* alleles, with 7 d.f.; B, heterogeneity between "backgrounds," with 1 d.f.; R, T-(A + B), with 7 d.f.

* χ^2 values having $P < 0.05$, by chance; ** χ^2 values having $P < 0.005$, by chance.

In eight of the ten cases where overall heterogeneity is evident, the component R contributes a significant portion of the total χ^2 . However, the proportion of χ^2 attributable to allele-specific differences (A) is often much greater than that attributable to interaction. Indeed, in at least two of the four blocks of data associated with each auxotroph, the ratio A:R (which each have 7 d.f.) is greater than 5. We conclude, therefore, that this analysis shows that, although the expression of a given allelic combination in a given growth regime is by no means constant, there is strong evidence for allele-specific differences. The qualitative nature of these differences is discussed in the RESULTS section.

It should be noted that our genetic substitution procedure was of limited resolution, so that we are formally unable to distinguish allele-specific differences from differences generated by modifiers in the segment of chromosome surrounding the *gual-pur1-ras* complex, between *lz* and *m*.

Our analysis is not designed to differentiate between variation due to experimental fluctuation and to background modifiers, except in respect to the substituted portion of the X chromosome. There is rather little evidence for large effects of the X chromosome; in only one case (*pur1*¹/*ras-l* on yeast-sucrose medium at 29°) is the χ^2 component attributable to "background" (B) significant in the absence of a substantial interaction component (R). In those cases where there is a significant B component, but also an R of comparable or greater significance, it is not legitimate to deduce a *bona fide* background effect. Autosomal modifiers should increase the residual (R) χ^2 value.

TABLE 8

A summary of the expression of *ras-l* mutations in heterozygous combinations with *gual*^u, *pur1*² and *ras*²

<i>ras-l</i> allele	Nonlethal mutation		
	<i>gual</i> ^u	<i>pur1</i> ²	<i>ras</i> ²
D12	Strong	Weak	Strong
F1	Strong	Weak	Weak
F19	Strong	Weak	Weak
H6	Strong	Strong	Strong
H25	Strong	Weak	Strong
J5	Weak	Strong	Strong
K27	Weak	Strong	Strong
N23	Strong	Weak	Strong

These qualitative assessments are based on data in Tables 3, 5 and 6. *pur1*¹ (Table 4) shows strong expression against all eight mutations.

So far, we have considered each *rasberry-lethal* mutation only as it affects each of the quasi-independent functions in the *gual-pur1-ras* complex. We now extend our analysis to the overall pattern of mutant expression and ask whether the variation observed in one function is correlated with that in other functions. Clearly it is not. Table 8 reduces the variation to its most basic form, by concentrating only on coarse differences in expression; each genotype is categorized either as "strong" or "weak." This arbitrary, but conservative, classification yields four different classes of mutation: those that affect only the *gual* function strongly (F1, F19), those that affect the *gual* and *ras* functions strongly (D12, H25 and N23), those that affect the *pur1* and *ras* functions strongly (J5 and K25) and, finally, one (H6) that affects all three functions strongly.

There is no consistent positive correlation between the strength of effect of each lethal mutation on *gual*, *pur1* and *ras*. It is, thus, impossible to explain the phenomenology in terms of a series of hypomorphs having different degrees of "leakiness," interacting with differing thresholds for the expression of each pleiotropic effect. Rather, they have to be viewed as a set of qualitatively different mutations generating largely independent levels of effect on the three functional domains within the complex. This picture is compatible not only with the existence of the three mutations, *gual*^u, *pur1*² and *ras*², which complement *inter se*, but also with the dual effect of *pur1*¹ on the *pur1* and *gual* functions.

CONCLUSION

We have studied eight EMS-induced recessive lethals in a single lethal complementation group associated with the *gual-pur1-ras* complex. We presume that these lethals are point mutations. In complementation tests with the nonlethal mutations *gual*^u, *pur1*² and *ras*, all but one recessive lethal prove to be allelic with all three nonlethals; the final lethal is allelic with *gual*^u and *pur1*².

Our findings confirm previous observations (NASH *et al.* 1981), which were made with one EMS-induced and two X-ray mutations, and confirm the presence of a complex locus. The nature of the lethals is highly variable. There are at least four clearly differentiable classes of mutations; it is possible to argue that all eight are different. This observation lends support to the contention that we have isolated point mutations, as opposed to deficiencies, that would be expected to be more monotypic in effect.

Complex genes are not uncommon in *D. melanogaster*, although satisfactory biochemical explanations for their genetic behavior remain rare. At present, the rudimentary locus (*r*, 1-54.5) provides the clearest model with which we can compare the *gual-pur1-ras* complex. There are two levels of phenotypic complexity of rudimentary. First, the phenotypic expression of its mutations includes three components; a wing defect, female sterility and pyrimidine auxotrophy. Expression of these three phenotypes is correlated in different alleles and appears to stem from the degree to which pyrimidine biosynthesis is defective (FALK and NASH 1974b); at this level there is one dimension to variation, and the differences between expression of different alleles are trivial.

At a second level, variation between *r* mutations is nontrivial. The locus encodes an enzymatically trifunctional polypeptide which catalyzes three reactions in pyrimidine biosynthesis (MEHL and JARRY 1978). It is this aspect of *r* mutations that generates complex genetic behavior, including an elaborate pattern of interallelic complementation (CARLSON 1971) which suggests that one, two, or all three enzyme activities may be reduced by a given mutation and that the severity of the effects on each activity is, at least, quasi-independent. Our results with the *gual-pur1-ras* region indicate variation of this latter kind.

Whether the explanation of our results is to be found in a multifunctional polypeptide is an open question. The results do not rule out the possibility. However, there is a distinction between our phenomenology and that exhibited by rudimentary that suggests other possibilities: the auxotrophic phenotypes themselves and, in particular, auxotrophy and the eye-color phenotype, do not appear to have *closely* related biochemical origins. Reduction in GTP-cyclohydrolase in *ras* mutants occurs autonomously in the head capsule and is not accompanied by a comparable effect in other tissues or at other developmental stages (EVANS and HOWELLS 1978). The lethality of *ras-l* mutants occur in larval life (NASH *et al.* 1981) and is cell-autonomous in all epidermal tissues (as indicated in somatic crossover tests by D. NASH, unpublished results). Thus, at present, there is some evidence that the several biological functions inferred to be encoded in the *gual-pur1-ras* region are not expressed together in a given cell. In contrast, it is difficult to envision how the three components of rudimentary function could be useful unless they were coordinately expressed.

Considering the large amount of evidence for the existence of complex loci, it remains puzzling that so very few instances of complex lethal loci have been described. NASH and JANCA (1983) have pointed out that the evidence is even less substantial than previously had been thought. In this report, we present substantial evidence that the *gual-pur1-ras* region does represent a *bona fide*

complex locus. It does yield recessive lethal mutations. Yet, paradoxically, it behaves as a simple locus when subjected to lethal complementation tests.

We suspect that the mutational spectrum that we have observed over the years provides a clue to this puzzling absence of complex lethal loci. We have found very few monofunctional mutations (two auxotrophs, and *no* viable *raspberry* alleles, in about 15,000 chromosomes screened). In contrast, multifunctional *ras* lethals occur at a high frequency (eight per 7,500 chromosomes in this study). A similar predominance of multifunctional defects at an hypothetical complex lethal locus would obscure its complex nature.

We suggest that essential loci are likely to be as functionally complex as nonessential loci; however, the operational test of functional complexity, the complementation test, is not necessarily as appropriate for the two cases.

Now that molecular tools are available that allow direct assessment of genetic complexity, it is likely that both particular questions about the organization and function of the *gual-pur1-ras* complex and more general questions suggested by our results will soon be answered.

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