SYNTHETIC LETHALITY AND SEMI-LETHALITY AMONG FUNCTIONALLY RELATED MUTANTS OF *DROSOPHILA MELANOGASTER*[1]

JOHN C. LUCCHESI

Department of Zoology, University of North Carolina, Chapel Hill, North Carolina

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THE sex-linked mutant *deep orange* (*dor*) of *Drosophila melanogaster* is responsible for a peculiar type of female sterility: mutant females produce no progeny when crossed to mutant males but yield some offspring (heterozygous daughters) when mated to wild-type males (MERRELL 1947). The effects of this mutant on embryonic development have been extensively studied by COUNCE (1956a) and more recently by HILDRETH and LUCCHESI (1967). In addition to the sterility phene, *dor* alters the pigmentation of the eyes (which are orange in color) and confers an abnormal spectrum of relative pteridine concentrations. An unusual characteristic of *dor* is that isoxanthopterin appears to be accumulated by mutant females (COUNCE 1957).

During the course of preliminary investigations into the biochemical basis of *dor* female-sterility, an attempt was made to prevent the accumulation of isoxanthopterin in mutant females by genetic means. This resulted in the discovery of a highly specific *synthetic lethal* system involving *dor* and a non-allelic third chromosome mutant, *rosy* (*ry*). The *dor-ry* system is similar to three other synthetic lethal systems, previously described in *D. melanogaster: purpleoid* (*pd*) and *Purpleoider* (*Pdr*) (BRIDGES 1922; as cited in BRIDGES and BREHME 1944); *prune* (*pn*) and *Prune-killer* (*K-pn*) (STURTEVANT 1956); and *Henna-recessive-3* (Hn^{r3}) and *rosy* (ry^{6}) (TAIRA 1960; GOLDBERG, SCHALET and CHOVNICK 1962).

A search was undertaken for the purpose of uncovering additional interactions among some of the mutants mentioned above. Since six of the latter affect eye color and/or pteridine levels, a number of other eye color mutants were tested. In addition, the female-sterile mutant *fused* (*fu*), similar in many respects to *dor* (although allowing lethal embryos to develop further than *dor* embryos (COUNCE 1956b)), was used in some crosses. The results of these investigations are presented in this paper.

MATERIALS AND METHODS

Biological data: The various mutant alleles with their genetic map locations and salient phenotypic characteristics are listed in Table 1 (for additional information the reader is referred to BRIDGES and BREHME 1944). The balancers used in the crosses were *ClB* (see BRIDGES and BREHME *op. cit.*) which is lethal when present in males, and M-5 (see SPENCER and STERN 1948) for the

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TABLE 1

Characteristics of mutants tested for viability interractions

Name	Symbol	Locus	Phenotype
deep orange	*dor*	X, 0.15	Eye color orange. Female-sterile.
fused	*fu*	X, 59.5	Wing veins fused at base. Female-sterile.
maroon-like	*ma-l*	X, 67.2	Eye color dull red.
clot	*cl*	2, 16.5	Eye color maroon.
cinnabar	*cn*	2, 57.5	Eye color bright scarlet.
brown	*bw*	2, 104.5	Eye color brownish to garnet.
purpleoid	*pd*	2, 106.4	Eye color dark pink.
sepia	*se*	3, 26.0	Eye color dark brown.
rosy	*ry*	3, 52.3	Eye color dull red.

X-chromosome; *Cy* for chromosome 2 and *Dcx* for chromosome 3 (BRIDGES and BREHME, *op. cit.*) which are lethal in homozygous condition. Mention must be made that the *Cy* balancer carries the recessive mutant allele cn^2.

The first series of crosses designed to test the interactions of *dor* with ry^1, ry^2, *bw*, *cn*, *cn bw*, *se*, or *cl* was performed by mating parents of the following generalized genotypes:

♀ *dor/Bal; m/m* × ♂ *dor*/Y; *Bal/m*

where "*Bal*" represents *ClB* and *Cy* or *Dcx*, and "*m*" represents one of the above mutants or the mutant combination of *cn bw*.

The second series of crosses was designed to test the interactions of *dor* with *pd*, and of *fu* with ry^2, *bw* or *pd*. The matings were:

♀ *dor*/+ or *fu*/+; *Bal/m* × ♂ *dor*/Y or *fu*/Y; *Bal/m*

"*Bal*" represents the same balancers as in the previous series and "*m*" the autosomal mutants listed above. Since these crosses yield comparable male and female progeny classes, they were used to retest *dor* with ry^2 and *bw*.

To study the possible interaction of *dor* and *ma-l*, a special series of crosses had to be devised since (1) *ma-l* is X-linked, and (2) it is subject to a maternal effect which confers a wild-type phenotype upon genotypically mutant progeny produced by heterozygous mothers. The following crosses were performed:

♀ *dor* Bx^3 *(ma-l)/Bal* × ♂ *dor* Bx^3 *(ma-l)*/Y.

The parentheses indicate that these parents were produced by heterozygous mothers; the males are, therefore, phenotypically wild-type as far as *ma-l* is concerned; this is true also of all offspring from such crosses. "*Bal*" represents M-5; Bx^3 is the dominant mutant *Beadex*, located at 59.4 and affecting wing shape. On the other hand, crosses involving:

♀ *dor* Bx^3 *ma-l*/+ Bx^3 *ma-l* × ♂ *dor* Bx^3 *(ma-l)*/Y

yielded non-maternally affected maroon-like progeny.

Six to ten pairs of parents were placed in standard culture bottles containing cornmeal-Brewer's yeast-molasses-agar medium; Tegosept-M and propionic acid were added as mold-inhibitors and the cultures were seeded with live yeast. After the females oviposited for a period of six days, the parents were removed. Daily counts of offspring were performed in some cases while in others the offspring were allowed to accumulate and were scored on the 16th to 18th day of culture. All crosses were incubated at 25 ± 1°C. In one case, the same general procedure was followed with the exception that pair matings were performed in vials.

Statistical analysis: If two mutants are acting independently of one another, their effect on the viability of a double homozygote may be expected to equal the product of the respective effects of each mutant. Taking independence as the null hypothesis, one can test whether two mutants interact in homozygous condition in such a fashion that they affect the viability to a greater extent than expected on the basis of their separate effects. Let h = number of double homozygotes, k = number of homozygotes for one mutant, H = number of homozygotes for the

other mutant, and K = number of double heterozygotes. Barring chance fluctuations, the null hypothesis states that: $h/K = k/K \cdot H/K$, i.e., $hK/Hk = 1$, or $\ln (hK/Hk) = 0$. WOOLF (1955) gave the variance of $\ln (hK/Hk)$, which is asymptotically normally distributed with mean zero. HALDANE (1956) modified this method of testing the null hypothesis to take account of small numbers in the sample; his statistic, which is used in this paper, is:

$$\chi = \frac{\ln \left[\frac{(2h+1)(2K+1)}{(2H+1)(2k+1)} \right]}{\sqrt{\frac{(H+h)(K+k)}{(H+K)(h+k)(H+K+h+k)}}}$$

Under the null hypothesis, the appropriate significance levels are obtained by referring to tables of the standard normal deviate.

RESULTS

The results of the first series of crosses, designed to test the interaction of *dor* with ry^1, ry^2, *bw*, *cn*, *cn bw*, *se*, or *cl*, are presented in Table 2. The data reveal no synergistic interaction between *dor* and *bw*, *cn* or *cl;* on the contrary, it would appear that *dor* flies homozygous for these mutants fare better than those of their sibs which are heterozygous, carrying a balancer chromosome.

Taking the double heterozygote class as unity, it is possible to calculate the reduction in viability, due to one or the other of the two mutants under consideration, among the female progeny of a given experiment. Considering the effect of the two mutants in the double homozygote as multiplicative, the data show that 0.8% and 6.5% of the expected *dor; cn bw* females were recovered in the bottle and vial experiments respectively; of the expected *dor; se* homozygotes, 89.0% were recovered.

The statistical test indicates that the null hypothesis should be rejected (i.e., there is a greater effect on the viability of the double homozygote than expected on the basis of a simple multiplicative relationship between the separate effects

TABLE 2

Results of crosses between dor/Bal;m/m *and* dor/Y;Bal/m

	Males		Females				
m	*dor*/Y; *Bal*/*m* 1*	*dor*/Y; *m*/*m* 1	*dor*/*dor*; *Bal*/*m* 1	*dor*/*dor*; *m*/*m* 1	*dor*/*Bal*; *Bal*/*m* 1	*dor*/*Bal*; *m*/*m* 1	N
ry^1	117	. . .	80	. . .	174	141	512
ry^2	170	. . .	150	. . .	208	154	682
bw	274	304	206	284	419	451	1,938
cn	172	241	181	233	535	472	1,834
cn bw (mass)	386	12	312	2	585	465	1,762
cn bw (vials)	220	21	238	15	316	308	1,118
se	301	183	230	187	404	369	1,674
cl	102	176	110	195	336	292	1,211

* Expected Mendelian ratios.

TABLE 3

Results of crosses between ♀ dor/+ *or* fu/+;Bal/m *and* ♂ dor/Y *or* fu/Y;Bal/m

	Males*				Females*				
m	*x*/Y; *Bal*/*m* 2†	*x*/Y; *m*/*m* 1	+/Y; *Bal*/*m* 2	+/Y; *m*/*m* 1	*x*/*x*; *Bal*/*m* 2	*x*/*x*; *m*/*m* 1	*x*/+; *Bal*/*m* 2	*x*/+; *m*/*m* 1	N
(1) *ry*^{2}	87	. . .	243	67	54	. . .	243	101	795
(2) *bw*	68	28	432	196	53	21	459	196	1,453
(3) *pd*	61	3	498	230	31	. . .	644	254	1,721
(4) *ry*^{2}	162	27	405	186	146	33	422	220	1,601
(5) *bw*	549	280	637	299	352	189	693	331	3,330
(6) *pd*	507	231	989	443	460	202	930	448	4,210

* "*x*" represents *dor* in crosses 1, 2, and 3; *fu* in crosses 4, 5, and 6.

† Expected Mendelian ratios.

of the two mutants) in the case of *dor* and *ry*^{1}, *ry*^{2}, *cn bw*, or *se*. The values of *x* and the associated significance levels are given in Table 5.

The results of the second series of crosses are presented in Table 3. These data confirm the synergistic interactions on viability of *dor* and *ry*, and reveal a similar effect for *dor* and *pd*, and for *fu* and *ry*^{2}. Minimal interactions were recorded, in these crosses, between *dor* and *bw*, in males, and between *fu* and *pd*, in females. The values of *x* and the frequency of recovery of double homozygotes as percentage of the expected number on the basis of a multiplicative effect of individual mutants, are given in Table 5.

The third series of crosses (see Table 4) clearly indicates the absence of demonstrable interaction between *dor* and *ma-l*, whether the latter is maternally affected or not. The discrepancy between the two female or the two male progeny classes, in both types of crosses, can be readily attributed to a halving of the viability of hemi- and homozygotes by the mutant *dor*.

DISCUSSION

In the following discussion, a *lethal* is defined as a factor which allows from 0% to 3% of the expected Mendelian class to emerge in culture; a *semi-lethal*

TABLE 4

Results of crosses designed to study the interaction of dor *with* ma-l

Males		Females		N
		Maternal effect present		
dor Bx^3*(ma-l)*/Y	*Bal*/Y	*dor Bx*^{3}*(ma-l)*/*dor Bx*^{3}*(ma-l)*	*dor Bx*^{3}*(ma-l)*/*Bal*	
102	154	95	191	542
		Maternal effect absent		
dor Bx^3 *ma-l*/Y	+ *Bx*^{3} *ma-l*/Y	*dor Bx*^{3} *ma-l*/*dor Bx*^{3} *ma-l*	*dor Bx*^{3} *ma-l*/+ *Bx*^{3} *ma-l*	
160	386	214	439	1,199

TABLE 5

Statistical analysis of pertinent progenies from Tables 2 and 3

	Mutant progenies	χ	P	Observed frequency in percent	Type of interaction
dor–ry^1	females (Table 2)	—81.25	$\ll 0.0001$	0	Synthetic lethal
dor–ry^2	females (Table 2)	—121.43		0	
dor–ry^2	females (Table 3)	—35.04		0	
	males	—85.45		0	
dor–bw	females (Table 3)	—1.11	$0.13 > P > 0.12$	95.5	None(?)
	males	—1.74	$0.045 > P > 0.040$	90.3	
dor–cn bw (mass)	females (Table 2)	—75.80	$\ll 0.0001$	0.8	Synthetic semi-lethal
dor–cn bw (vials)	females (Table 2)	—75.45		6.5	
dor–se	females (Table 2)	—3.83	< 0.0006	89.0	None(?)
dor–pd	females (Table 3)	—39.48	$\ll 0.0001$	0	Synthetic semi-lethal
	males	—35.26		9.4	
fu–ry^2	females (Table 3)	—21.15		43.4	Synthetic semi-lethal
	males	—20.44		36.5	
fu–pd	females (Table 3)	—4.17	< 0.0006	94.4	None(?)
	males	>0	....	94.3	

is one which allows from 4% to 49% of the expected Mendelian class to emerge; and a *subvital* factor is one which allows recovery of at least 50% but less than 100% of the expected class (these definitions are consistent with those of HADORN 1955). The term *synthetic lethal* was coined by DOBZHANSKY (1946) to describe complementary lethal systems in wild-type populations of *D. pseudoobscura*: synthetic lethality was obtained by allowing two homologous chromosomes of different origin, perfectly viable as homozygotes, to recombine; certain genes, born by each original chromosome, were now on the same chromosome and interacted to produce a recessive lethal effect.

Within the limits of the above definitions, the combination of the mutants *dor* and *ry* constitutes a synthetic lethal system. Furthermore, the data suggest that *dor* with *cn bw*, *dor* with *pd*, and *fu* with *ry* represent synthetic semi-lethals. The nature of the experimental design and material do not permit judicious assessment of some subvital interactions which are indicated among various mutants; no commitment in this respect is, therefore, made in Table 5.

The synthetic lethal interaction of *dor* with *ry* appears to be highly specific since *dor* is perfectly viable in combination with *ma-l*. Both *ry* and *ma-l* flies lack xanthine dehydrogenase activity (GLASSMAN and MITCHELL 1959a); yet the two mutant sites are located on different chromosomes, *ma-l* is subject to a maternal

effect by its wild-type allele, and *ma-l* flies produce "cross-reacting material" (GLASSMAN and MITCHELL 1959a and 1959b); additional differences between *ry* and *ma-l* have been reported by FORREST, HANLY and LAGOWSKI (1961). Among these differences must lie the reason for the differential response of *dor* with these two mutants.

The synthetic lethal interaction of *dor* with *ry* is temperature sensitive. This fact was brought to the attention of the author by Prof. C. W. CLANCY who succeeded in obtaining *dor;ry* double homozygotes by performing the appropriate crosses at low temperatures. The temperature dependence of the lethal interaction is demonstrated by Prof. CLANCY's unpublished data, presented in Table 6. Mention must be made that *ry* mutants alone have been shown to exhibit semi-lethality at 29°C. (CHOVNICK, SCHALET, KERNAGHAN, and TALSMA 1962).

The mutant *ry* yields a synthetic semi-lethal effect when present in flies which are hemi- or homozygous for the female-sterile mutant *fu*. Mention must be made that the latter shares at least one phenotypic characteristic with *ry*: although the eye pigmentation of *fu* flies seems relatively normal, the relative pteridine concentrations found in these flies appear to differ from those found in wild-type (S. J. COUNCE, personal communication).

TAIRA (1960) reported that the double mutant *Henna-recessive-3* (Hn^{r3}, an eye color mutant located at 23 on the third chromosome) and *ry* constitutes a lethal. Subsequent work by GOLDBERG, SCHALET and CHOVNICK (1962) showed that this specific interaction is obtained with one of the *ry* alleles: ry^6; combinations of Hn^{r3} with ry^1, ry^2, ry^4 and ry^9 proved viable. GOLDBERG *et. al.*, performed chromatographic examinations of pteridines in the various genotypes involved in their crosses. Of considerable interest is their observation that, whereas double heterozygotes for Hn^{r3} and *ry* exhibited qualitatively normal pteridine spectra, all viable mutant homozygotes and all combinations of *ry* heterozygotes (homozygous for Hn^{r3}) appeared to accumulate biopterin and 2-amino-4-hydroxy-pteridine and to lack isoxanthopterin. As previously stated, the latter pteridine is not synthesized by *ry* homozygotes and is accumulated by *dor* females.

The combination of *dor* and *pd* is semi-lethal. The latter mutant is part of the earliest synthetic lethal system on record in Drosophila. BRIDGES (1922) observed that this semi-dominant mutant was lethal, when present in double homozygotes,

TABLE 6

Temperature sensitivity of dor–ry *synthetic lethality: results of crosses between* ♀ dor/Bal;ry/ry × ♂ dor/*Y*;ry/ry*

Culture incubation temperature	Males	Females		N
	dor/Y;ry/ry	*dor/dor;ry/ry*	*dor/Bal;ry/ry*	
25°C	42	52	1,618	1,712
22°C	1,151	944	1,399	3,494
18°C	520	374	538	1,432

* The data were kindly supplied by DR. C. W. CLANCY. The parents were obtained at 22°C.

with the dominant mutant *Purpleoider* (*Pdr*, an eye color mutant located at 46 on the third chromosome).

Another semi-lethal system, constituted by combining *dor* with *cn* and *bw*, was uncovered. This effect is underscored by the normal viability of *dor* with either of the two mutants, separately. Of interest here is the implied functional relationship between two different biosynthetic pathways: that of the pteridines (blocked by *bw*) and that of the ommochromes (blocked by *cn*). Such a relationship has, in the past, been inferred by a number of workers.

Finally, the highly specific lethal system described by STURTEVANT (1956) remains to be discussed. Flies hemi-or homozygous for the sex-linked recessive eye color mutant *prune* (*pn*) are not recovered if the dominant autosomal mutant *Prune-killer* (*K-pn*, at 104.5 on chromosome 3) is present in the genotype. This mutant is dominant solely with reference to its interaction with *pn* since it appears to have no other distinguishable phenotypic characteristic. STURTEVANT confirmed the killer effect of *K-pn* with several *pn* alleles. Furthermore, he unsuccessfully attempted to modify the synthetic lethality by introducing various mutants (all affecting pigment synthesis or deposition) into the system. Among the mutants tried were *cn*, *bw*, *se*, *pd*, *vermilion* (*v*), *scarlet* (*st*), *white apricot* (w^a), *white eosin* (w^e), *zeste* (*z*), *claret* (*ca*) and *chocolate* (*cho*).

It is quite obvious that the various genotypes discussed above constitute but a small fraction of the total possible double homozygotes which can be synthesized using the genes which these genotypes represent. Furthermore, if one considers all of the mutants known to affect eye pigment synthesis and their numerous alleles, in addition to one or two more female-sterile mutants, the number of possible permutations staggers the imagination and their synthesis lies beyond the realm of practical endeavour. Nevertheless, the seven specific synthetic interactions uncovered to date, involving a group of 10 mutants, warrant the following considerations. With the possible exception of *K-pn*, all of the mutants are known to confer upon the flies an abnormal pteridine spectrum and are, therefore, functionally related. The basis of lethality may, in turn, be related to pteridine metabolism. The seven synthetic lethal and semi-lethal systems may represent cases where the action of the mutants involved affects pteridine metabolism in such specific complementary fashion that lethality ensues; perhaps not directly, since pteridines *per se* do not appear to be essential metabolites in laboratory stocks of Drosophila (witness the range of pteridine levels exhibited by all the viable eye color mutants), but as a secondary effect, an interference with some essential metabolic pathway. The same metabolic block may be involved in the female-sterile mutants where lethality would be delayed for a generation. This working hypothesis is currently under investigation in the author's laboratory.

I wish to thank Professor C. W. CLANCY for kindly making the data on temperature sensitivity available to me and for allowing me to include them in this paper. I also wish to thank Professor R. E. ELSTON for his generous aid with the statistical treatment of the data.

SUMMARY

The female-sterile mutant *deep orange* (*dor*) has been found to exhibit a

"synthetic lethal" effect when present in combination with *rosy* (*ry*), and a "synthetic semi-lethal" effect in combination with *cinnabar* (*cn*) and *brown* (*bw*), or with *purpleoid* (*pd*). In addition, the female-sterile mutant *fused* (*fu*) exhibits semi-lethality in combination with *ry*.—Some of the above mutants (*ry*, *pd*) are involved in two of three synthetic lethal systems previously described by different authors.—A common biochemical basis for lethality in the above systems and in the progeny of female-sterile flies, indirectly related to pteridine biosynthesis, is proposed.

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