THE EFFECT OF LARVAL INTERACTION ON VIABILITY IN *DROSOPHILA MELANOGASTER*. I. CHANGES IN HETEROZYGOSITY

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THE persistence of the mutant gene ebony (*e*) in experimental populations of *Drosophila melanogaster*, first demonstrated by L'HERITIER and TESSIER (1937), has generally been ascribed to the superiority of ebony/wild-type heterozygotes. Investigations that have supported this view include measurements made of the effects of temperature and humidity changes (KALMUS 1945), larval crowding (MOREE 1952; MOREE and KING 1961) and mating success (JACOBS 1961) on cultures segregating for the three genotypes, $+/+$, $+/e$ and e/e . However, since the wild-type stocks used in these studies were mostly derived from single strains, only few wild-type isoalleles may have been segregating among the wild-type homozygotes in the test cultures. If superior performance depends upon heterozygosity at many loci, then the poor performance of wild-type homozygotes in these cases may have been caused by their relatively high degree of homozygosity compared to the $+/e$ heterozygotes that were at least heterozygous for genes near the ebony locus. The most direct evidence for this view is that of POLIVANOV (1964) who demonstrated that the greater the number of different wild-type stocks introduced into population cages of competing ebony/wild-type genotypes, the more rapid the decline in ebony frequency.

As a further test of the effect of wild-type heterozygosity on the viability of ebony, the present experiment measured the effect of different wild-type strains, each with different inbreeding histories, on single generation cultures segregating for the three genotypes, $+/+$, $+/e$, and e/e , in a ratio whose expected values are 1:2:1. The tests consisted of competition between a strain of ebony and each wild-type strain individually ("intrastrain crosses") as well as competition between ebony and two of these wild-type strains at the same time ("interstrain crosses"). Each test was repeated at two different temperatures 25°C and 18°C, and also at a fluctuating diurnal temperature of 25°–18°C.

MATERIALS AND METHODS

The ebony mutant stock used was a laboratory strain of e^{11} long maintained by mass transfer. The three different wild-type strains had the following origins: (1) An Oregon-R strain designated as " $+^0$," provided by Amherst College, that had been maintained by close inbreeding

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for about 380 generations. (2) A strain designated as "+^J" originally captured in Berkeley, California, in 1949 by E. JUND, and maintained since then in half-pint culture bottles by mass transfer. (3) A strain designated as "+^B" captured in Berkeley in June 1962 immediately prior to the present experiments. From each of these three wild-type strains 20 males were crossed to 20 virgin ebony females and transferred at two-day intervals to new culture bottles. (From strain +^B only captured males were used.) F₁ offspring of these matings, all heterozygous for ebony and wild-type alleles, were allowed to age for three days and then mated *inter se* on the fourth day in the following fashion:

Intrastrain crosses

$$+^O/e \times +^O/e$$

$$+^J/e \times +^J/e$$

$$+^B/e \times +^B/e$$

Interstrain crosses

$$+^O/e \times +^J/e$$

$$+^O/e \times +^B/e$$

$$+^J/e \times +^B/e$$

Each of these intra- and interstrain crosses consisted of 20 pairs of F₁ flies placed in a half-pint culture bottle in six replications. For each of the interstrain crosses, three replicates were composed of males of one F₁ strain and females of the other, while the other three replicates were reciprocal matings of these. After a 24-hr period of oviposition, the six replicate groups for each cross were transferred to new cultures for a similar length of time. This second period of oviposition was then followed by a further day-long transfer, after which the parents were discarded. Thus, each of the above crosses furnished three sets of egg-containing cultures which were then handled in the following fashion: (1) Cultures from the first 24-hr egg-laying period were placed at 25°C constant temperature. (2) Cultures from the second day of egg-laying were kept at a diurnal fluctuating temperature of 18 hours at 25°C and 6 hours at 18°C. (3) Cultures from the third day of egg-laying were placed at 18°C constant temperature.

Cornmeal-molasses-agar was used as culture medium with propionic acid added as mold inhibitor. Immediately prior to the introduction of F₁ parental flies, each culture was inoculated with one drop of an aqueous suspension of Fleischmann's Active Dry Yeast and a uniform strip of paper toweling was added.

The emerging F₂ flies were counted daily and scored phenotypically as ebony or wild type. In order to discern the respective frequencies of +/e and +/+ genotypes, a total of about 40 to 50 emerging F₂ wild-type males was taken from each culture during the first three counts. These males were then mated in single pairs with homozygous ebony females and their genotypes scored according to the phenotypes of their progeny.

RESULTS

Each F₁ cross between ebony/wild-type heterozygotes would be expected to produce F₂ offspring in the ratio of 1+/+: 2+/e: 1e/e, or phenotypically in the ratio of 3 wild type: 1 ebony. Table 1 presents the observed phenotypic frequencies as well as the chi-square values for a 3:1 ratio in each cross.

For the intrastrain crosses, chi-square values show little statistical significance for reduced ebony frequencies except at the 25°-18° fluctuating temperature in which the +^J and +^B crosses are within the borderlines of significance. For six of the interstrain crosses, ebony frequencies are significantly reduced; extremely high chi-square values are observed at 25°-18° and at 18°, all with probabilities less than .001.

Accompanying the reduced frequencies of ebony at these last two temperatures is a marked increase in numbers of F₂ progeny produced by each cross. This increase occurs in both intra- and interstrain crosses but is distinctly higher in the latter group. Apparently, the factors which reduce the frequency of ebony homozygotes are strongly associated with numbers of larvae per culture, a finding

TABLE 1

Number and frequency of wild-type and ebony phenotypes in the F_2 progeny of intra- and interstrain crosses at different temperatures

F_1 cross	Temperature ($^{\circ}\text{C}$)	F_2 phenotypes		Total	Percent e/e
		+/-	e/e		
$+^0/e \times +^0/e$	25	1535	503	2038	24.68
$+^J/e \times +^J/e$	25	2189	682	2871	23.75
$+^B/e \times +^B/e$	25	2086	713	2799	25.47
$+^0/e \times +^0/e$	25-18	4178	1339	5517	24.27
$+^J/e \times +^J/e$	25-18	4031	1227	5258	23.34**
$+^B/e \times +^B/e$	25-18	4409	1377	5786	23.80*
$+^0/e \times +^0/e$	18	4004	1303	5307	24.55
$+^J/e \times +^J/e$	18	3237	1083	4320	25.07
$+^B/e \times +^B/e$	18	3773	1256	5029	24.98
$+^0/e \times +^J/e$	25	1392	474	1866	25.40
$+^0/e \times +^B/e$	25	1485	471	1956	24.08
$+^J/e \times +^B/e$	25	1518	500	2018	24.78
$+^0/e \times +^J/e$	25-18	4867	1427	6294	22.67***
$+^0/e \times +^B/e$	25-18	5482	1539	7021	21.92***
$+^J/e \times +^B/e$	25-18	5254	1340	6594	20.32***
$+^0/e \times +^J/e$	18	4885	1324	6209	21.32***
$+^0/e \times +^B/e$	18	5066	1330	6396	20.79***
$+^J/e \times +^B/e$	18	4585	1317	5902	22.31***

* $P < 0.05$; ** $P < 0.01$;

*** $P < 0.001$ for chi-square testing deviation from 3:1. Heterogeneity chi-squares are all nonsignificant.

that has been well documented by MOREE and KING (1961). In the present experiments, at least two possible causes may be responsible for this increase in numbers at the cooler temperatures. First, the number of eggs laid may have been the same in each cross but viability at the cooler temperatures is improved over that at 25° . Secondly, the egg-laying pattern may have been such that fewer eggs were laid the first day after etherization and mating (cultures placed at 25°) and more eggs laid on the second and third days (cultures placed at the cooler temperatures). Although the present data do not allow discrimination between these possibilities, superficial observation of the cultures did not show any observable increase in larval or pupal mortality at 25° . Furthermore, egg-laying patterns with successive increases to peak values have been demonstrated many times (HANSON and FERRIS 1929; KAUFMANN and DEMEREC 1942; ROBERTSON and SANG 1944; and others) and may well account for the disparity in numbers between days.

Whatever the cause for this difference, it is of special interest that the increase in numbers at the cooler temperatures is higher among the interstrain crosses and also leads to a greater reduction in ebony frequency relative to the intrastrain crosses. Since the F_1 parents for both intra- and interstrain crosses were derived

from the same cultures and were aged the same length of time, the difference in F_2 numbers between them is most likely caused in this case by a difference in viability of the F_2 offspring. Thus, although interstrain crosses can be considered to produce offspring with increased viability, much of this increase appears to represent an improved fitness of wild-type phenotypes rather than of ebony homozygotes; of the 7,199 differential increase in number of F_2 offspring in the interstrain crosses at cooler temperatures, only 692 or 9.6 percent represent an increase in the number of ebony homozygotes.

Results of the 5,000 single-pair matings that were undertaken to test the genotypes of phenotypically wild-type males are presented in Table 2. With only two exceptions, the segregation of genotypes follows the expected ratio of $1+/:2+/:e$. The two exceptions do not show any pattern but are distributed between both intra- and interstrain crosses at two different sets of temperature. A marked trend, however, appears when the numbers of each of these two genotypes are gathered according to the degree of crowding in the cultures; the proportion of $+/+$ decreases from .364 under uncrowded conditions (25°) to .323 under crowded conditions (25° – 18° and 18°). In a chi-square test for independence this difference is significant at the .01 level (column I, Table 3). Further analysis reveals that

TABLE 2

Number and frequency of $+/+$ and $+/e$ males among samples taken from the F_2 progeny of intra- and interstrain crosses at different temperatures

F_1 cross	Temperature ($^\circ\text{C}$)	F_2 genotypes		Total	Percent $+/+$
		$+/+$	$+/e$		
$+^O/e \times +^O/e$	25	90	186	276	32.61
$+^J/e \times +^J/e$	25	100	172	272	36.77
$+^B/e \times +^B/e$	25	99	155	254	38.98
$+^O/e \times +^O/e$	25–18	91	199	290	31.38
$+^J/e \times +^J/e$	25–18	84	180	264	31.82
$+^B/e \times +^B/e$	25–18	92	177	269	34.20
$+^O/e \times +^O/e$	18	78	204	282	27.66
$+^J/e \times +^J/e$	18	74	213	287	25.78**
$+^B/e \times +^B/e$	18	116	206	322	36.03
$+^O/e \times +^J/e$	25	92	157	249	36.95
$+^O/e \times +^B/e$	25	87	149	236	36.86
$+^J/e \times +^B/e$	25	74	127	201	36.82
$+^O/e \times +^J/e$	25–18	123	191	314	39.17*
$+^O/e \times +^B/e$	25–18	111	187	298	37.25
$+^J/e \times +^B/e$	25–18	81	207	288	28.12
$+^O/e \times +^J/e$	18	91	213	304	29.93
$+^O/e \times +^B/e$	18	99	179	278	35.61
$+^J/e \times +^B/e$	18	94	219	313	30.03

* $P < 0.05$; ** $P < 0.01$; for chi-square testing deviation from 1:2. Heterogeneity chi-squares are nonsignificant except for $+^J/e \times +^B/e$ ($P < 0.05$) and $+^O/e \times +^J/e$ ($P < 0.01$).

TABLE 3

Relationship between the degree of crowding and the proportions of the two wild-type genotypes in the F_2 samples

	I Intra- and interstrain combined		II Intrastrain only		III Interstrain only	
	+/+	+/ <i>e</i>	+/+	+/ <i>e</i>	+/+	+/ <i>e</i>
Uncrowded (25°)	542	946	289	513	253	433
Crowded (25°–18°, 18°)	1134	2375	535	1179	599	1196
Contingency χ^2	7.72**		5.55*		2.56	

* $P < 0.05$.** $P < 0.01$.

the main contribution towards the fall in frequency of +/+ upon crowding occurs in the intrastrain crosses (column II) while the interstrain crosses show no significant difference in these values (column III).

Table 4 presents a genotypic analysis of the same wild-type males as in the previous two tables, but classified according to the three periods at which these males were extracted from the cultures. These periods were approximately 24 hours apart beginning with the first F_2 count, and offer some measure for comparing the developmental rate of +/+ and +/*e*. With one exception (first count, 18°) there is no significant difference between the developmental rates of these genotypes in intra- and interstrain crosses. As expected, however, there is a significant difference between uncrowded and crowded conditions (as defined above) in the rates at which the two genotypes appear. Each of the first two counts shows a highly significant increase in the proportion of +/*e* upon crowding, while the third count shows no such significant change. As shown in Table 5, when the counts are separated into intra- and interstrain groups, significant increases in the proportion of +/*e* occur only in the first two counts of the intrastrain crosses.

Table 5 also provides the opportunity for demonstrating that the drop in fre-

TABLE 4

*Numbers of +/+ and +/*e* males in the first, second and third samples taken from F_2 progeny of intra- and interstrain crosses at the three different temperatures*

Temperature	1st count		2nd count		3rd count	
	+/+	+/ <i>e</i>	+/+	+/ <i>e</i>	+/+	+/ <i>e</i>
Intrastrain	88	179	119	167	82	165
Interstrain	82	150	123	195	48	88
Contingency χ^2	.22		.42		.09	
Intrastrain	48	153	93	229	126	174
Interstrain	76	167	110	203	129	215
Contingency χ^2	2.63		2.58		1.18	
Intrastrain	57	234	106	212	105	177
Interstrain	76	179	100	210	108	222
Contingency χ^2	7.15**		.04		1.17	

** $P < 0.01$.

TABLE 5

Relationship between the proportions of the two wild-type genotypes and the degree of crowding for each of the three F₂ samples

	1st count		2nd count		3rd count	
	+/+	+/e	+/+	+/e	+/+	+/e
Intra- and interstrain combined						
Uncrowded (25°)	170	329	242	362	130	253
Crowded (25°–18°, 18°)	257	733	409	854	468	788
Contingency χ^2	10.29**		10.28**		1.26	
Intrastrain only						
Uncrowded (25°)	88	179	119	167	82	165
Crowded (25°–18°, 18°)	105	387	199	441	231	351
contingeny χ^2	11.71***		9.23**		2.83	
Interstrain only						
Uncrowded (25°)	82	150	123	195	48	88
Crowded (25°–18°, 18°)	152	346	210	413	237	437
Contingency χ^2	1.48		2.06		.003	

P<0.01. * P<0.001.

quency of +/+ upon crowding is notably different between intra- and interstrain crosses. If we confine our attention to those counts (1st and 2nd) which are associated with significant changes in frequency of the two wild-type genotypes upon crowding, we may note that for each of these counts the +/+ frequency falls more than 10 percent in intrastrain crosses, while it falls only 5 percent in interstrain crosses, or a ratio of 2:1.

DISCUSSION

The present experiments were designed to investigate the effect of different wild-type stocks on the frequencies of F₂ genotypes segregating from a cross between F₁ ebony/wild-type heterozygotes. Three wild-type strains (+⁰, +^J, +^B) were crossed with a single ebony mutant strain so as to enable intrastrain (+⁰/+⁰, +^J/+^J, +^B/+^B) and interstrain (+⁰/+^J, +⁰/+^B, +^J/+^B) effects to be distinguished. Cultures placed at 25° were either not as viable embryonically or had fewer eggs initially than those placed at two other temperatures (25°-18° and 18°). The data can therefore be grouped according to the degree of larval crowding in the cultures with little or no crowding at 25° and relatively high crowding at 25°-18° and 18°.

As shown in Table 1, the increased crowding at the lower temperatures is associated with decreased frequencies of ebony homozygotes in interstrain crosses but has relatively little effect in intrastrain crosses. This can be interpreted to mean that the fitness of the F₂ wild-type genotypes is enhanced by the increased heterozygosity of the interstrain combinations. Thus, although +^J and +^B are presumably more heterozygous than +⁰, each of these strains individually may be considered to be less heterozygous than any of the interstrain combinations. The viability manifestations of this increased heterozygosity in interstrain crosses, however, does not appear to be confined to either the +/+ or +/e genotypes. As

shown in Table 2, there is no significant difference from the expected ratios of $+/+$: $+/e$ in almost every combination. Since $+/e$ genotypes in all crosses are relatively constant in respect to heterozygosity at the ebony locus, their increased viability in interstrain crosses, and perhaps that of $+/+$ as well, may be caused by a general increase of heterozygosity at other loci besides ebony.

Support for this view can be obtained when the data of $+/+$ and $+/e$ frequencies are grouped according to the degree of crowding. As shown in Table 3, there is no significant difference between $+/+$ and $+/e$ proportions in the interstrain crosses upon crowding and noncrowding. For intrastrain crosses, however, there is a significant fall in the frequency of $+/+$ with crowding. In other words, heterozygosity at the ebony locus does not seem to matter in interstrain crosses where it is generally high for other loci, but is definitely advantageous in intrastrain crosses where it is lower for other loci. This type of epistatic interaction or "synergism" between the deleterious effects of a homozygote at one locus and recessives at other loci would not be unexpected (see MULLER 1950; SPIESS 1958; DOBZHANSKY and SPASSKY 1960; KRIMBAS 1960; and others).

An alternative explanation has been suggested by PROFESSOR E. R. DEMPSTER which relies upon the heterozygosity that arises from crossing over or its absence in the F_1 females. If we consider the chromosome sections at the ebony locus, then the proportion of heterozygosity for intra- and interstrain F_2 genotypes is as in Table 6. It is evident that if crossing over is either absent or even-numbered in the third chromosome, carriers of the $+/e$ genotype would be considerably more heterozygous than $+/+$ in the intrastrain crosses, but no more, or even less, heterozygous than $+/+$ in the interstrain crosses. If improvement in viability is proportional to the degree of heterozygosity, then the increase in the $+/e$: $+/+$ ratio upon crowding in intrastrain crosses is readily explained. On the other hand, according to this explanation, we might also expect a relative decrease in $+/e$: $+/+$ ratio in interstrain crosses upon crowding because of the relatively lower heterozygosity in the $+/e$ genotype. The actual data, however, show that the $+/e$: $+/+$ ratio is increased in interstrain crosses although not to the level of statistical significance. A clear demonstration of the validity of either of these two hypotheses cannot be made in the present experiment, and it is even possible that both explanations may apply.

TABLE 6

The proportion of heterozygosity at and near the ebony locus in the progeny of intra- and interstrain crosses

Genotype	Intrastrain				Interstrain			
	At <i>e</i>	Beyond 1st crossover	Beyond 2nd crossover	Average	At <i>e</i>	Beyond 1st crossover	Beyond 2nd crossover	Average
<i>e/e</i>	0	1	0	.33	0	1	0	.33
$+/e$	1	0	1	.67	1	$\frac{1}{2}$	1	.83
$+/+$	0	1	0	.33	1	1	1	1.00

The calculations are based on the apposition of genes from different strain origins, when crossover chromosomes of the F_1 $+/e$ female are combined with noncrossover chromosomes of the F_1 $+/e$ male.

In any case, the rare occurrence of ebony in natural populations as compared to its notable frequency in many laboratory populations may be explained as caused by differences in the viability of $+/e$ genotypes. In the former populations, the large number of individuals heterozygous at ebony and at many loci other than ebony offer little special advantage to the $+/e$ heterozygote. In the more confined and homozygous laboratory populations, the viability of the $+/e$ individual is improved because it is now comparatively more heterozygous. Such conclusions are also indicated by the work of POLIVANOV (1964).

Although the components of fitness improved by the heterozygosity of $+/e$ in the intrastain crosses in these experiments are not exactly known, they probably occur during larval competition since it is at this stage that an increase in crowding would most likely exert its greatest effect. As shown in Table 5, these larval advantages seem to be associated with more rapid development. To investigate this problem further, a second experiment (manuscript in preparation) has measured the viabilities of genotypes under conditions in which their relative ages during larval competition have been controlled.

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SUMMARY

Viability studies on single-generation cultures segregating for ebony have shown that the frequency of ebony homozygotes falls with increased degree of larval crowding and with increased heterozygosity of the wild-type strains present in the cultures. In this competition, the frequency of the $+/e$ genotype is increased relative to $+/+$ only when both crowding and homozygosity of the wild-type strains are relatively high. Competition in which there is increased heterozygosity at ebony and at loci other than ebony shows no increase in the $+/e$ genotype upon crowding. When present, the viability advantages of the $+/e$ genotype seem to be associated with early development.

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