THE EFFECT OF LARVAL INTERACTION ON VIABILITY IN *DROSOPHILA MELANOGASTER*. II. CHANGES IN AGE STRUCTURE

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THE degree of heterozygosity carried by competing genotypes in single generation cultures of *Drosophila melanogaster* segregating for ebony (e^{11}) and wild type has been shown to be a factor affecting their relative fitnesses (DAWOOD and STRICKBERGER 1964). Since these results also indicated that the fitness advantages of at least one of the segregating genotypes ($+/e$) was associated with rapid development, the present experiments set out to explore the effects caused by different larval ages among competing genotypes.

MATERIALS AND METHODS

Two wild-type stocks of *D. melanogaster*, the F_1 between them, and an ebony mutant stock were used in these experiments. One of the wild-type stocks, A2, was derived from a California strain made isogenic in 1948 and maintained by mass transfer in culture bottles in DR. C. STERN's laboratory at the University of California in Berkeley. The second wild-type strain, A6, was derived from a wild-caught Samarkand strain similarly maintained. The ebony mutant stock was a laboratory strain of e^{11} long maintained by mass transfer.

Larvae of desired genotypes and ages were obtained from caps containing agar-cornmeal-molasses medium that had been tightly fitted to half-pint bottles containing about 50 pairs of four-day-old adult flies. The caps were permitted to remain in the oviposition chambers for 24 hrs at 25°C and then removed and placed into previously sterilized cans. After 4-6 hrs in these cans, the freshly hatched larvae were collected by gentle adhesion to a dissecting needle. These larvae were designated by their approximate postfertilization age as "24 hrs" old. To obtain older larvae, the caps were kept in the cans for an additional 24 hrs before collection, and the larvae then collected were designated as "48 hrs" old.

All experiments testing larval viability were performed at 25°C in 25 mm × 95 mm shell vials containing 5 cc of sucrose-agar medium (KALMUS 1943) to which was added 5 mg of fresh live yeast dissolved in .1 cc of water. Larvae competing together in a single vial were all inserted as rapidly as the larval collection method permitted, usually within 5 min. The tests determining the viability of each genotype in the absence of competing genotypes were designated as "pure" cultures, and were performed at densities of 40 and 80 larvae per vial. At the lower density the larvae used were all 24 hrs old. At the higher density, one group of tests was performed in which all larvae were of the same age (24 hrs old), herewith designated as "80 equal ages." Another group of tests were performed in which 40 larvae in each vial were 24 hrs older (48 hrs old) than the other 40 (24 hrs old), herewith designated as "80 unequal ages." Twenty replicate vials were made at each density and for each different age structure. At the higher density, counts of emerging adults were made daily to enable comparisons among average developmental times of the different genotypes.

Tests of competition between two different genotypes ("mixed" cultures) were made by using larvae from the ebony stock as a common competitor with either A2, A6, or the F_1 of A2 × A6.

In this case, only densities of 80 larvae per vial were used (that is, 40 of each genotype), and the tests were performed at both equal and unequal age structures. Again, 20 replicates were made for each test and emerging flies were counted daily to determine the effect of interstrain competition on developmental period.

RESULTS

The results of viability tests in pure cultures are shown in Table 1. It is clear that the four genotypes have somewhat similar viabilities at densities of 40 larvae per vial, although t tests show that the A2 and F_1 viabilities are significantly different from those for the A6 and ebony genotypes. (A2 differs from A6 and ebony at the 5% level of significance, and F_1 differs from A6 and ebony at the 0.1% level.) At densities of 80 larvae per vial there is a marked drop in the percentage survival of all genotypes. This drop is especially obvious in the case of A6, in which only 410 adults emerged at 80 equal ages and 580 adults emerged at 80 unequal ages out of totals of 1600 larvae, compared to 693 that emerged out of only 800 larvae at the 40 density. Generally, the relative viabilities of the four genotypes at densities of 80 are not predictable from the results observed at densities of 40, although, with the exception of ebony, there is some consistency in the fact that viabilities at 80 unequal ages are greater than at 80 equal ages. At 80 equal ages, A6 reaches its lowest viability, whereas the viability of ebony is increased relative to A2 and F_1 , although not significantly so. At 80 unequal ages, A2 and F_1 are superior in viability to A6 and ebony as at the 40 density, but the highest viability is now possessed by A2. At both 80 equal and unequal age structures, the viability of A6 is significantly lower than all others.

Average developmental time for the four genotypes in pure cultures for both 80 equal and unequal ages is given in Table 2 and the daily emergence patterns are shown in Figure 1. There is obviously no correlation between average viability and average developmental time of genotypes ($r = -.13$, and the probability of the null hypothesis that $\rho = 0$ is more than .7). As expected, however, there is a general increase in developmental time in the tests at unequal age structure since developmental time is scored from the egg stage of the older larvae and would, therefore, include an ostensible increase of one day in the developmental

TABLE 1

Larval viabilities of the four genotypes in pure cultures at given densities and age structures

Density	Age structure		Genotypes			
			A2	A6	F ₁	ebony
40	equal	Percent survival	91.50	86.63	93.88	86.13
		mean number/vial	36.60±0.62	34.65±0.63	37.55±0.34	34.45±0.52
80	equal	Percent survival	65.88	25.63	60.69	69.75
		mean number/vial	52.70±1.02	20.50±1.88	48.55±1.78	55.80±3.21
80	unequal	Percent survival	72.31	36.25	62.13	46.69
		mean number/vial	57.85±2.06	29.00±1.77	49.70±2.18	37.40±2.65

Percent survival is based on the total number of adults expected to emerge from the 20 vials used in each test, i.e. 800 at densities of 40, and 1600 at densities of 80.

TABLE 2

Mean egg-to-adult developmental period in days for the four genotypes in pure cultures in densities of 80 larvae per vial at the two age structures

Genotype	80 equal ages	80 unequal ages
A2	12.42 $\pm$ 0.03	12.07 $\pm$ 0.04
A6	11.53 $\pm$ 0.05	14.23 $\pm$ 0.08
F ₁	13.08 $\pm$ 0.06	14.26 $\pm$ 0.06
ebony	11.36 $\pm$ 0.04	13.36 $\pm$ 0.07

time of the younger larvae. Similarly, the bimodal distribution of the emergence pattern at the unequal age structure can be considered to represent peaks caused by the separate emergence of the older and younger larvae, although this is more clearly shown in the mixed cultures when the age groups can be phenotypically differentiated. Surprisingly, in the case of A2, average developmental time is

FIGURE 1.—Percentages of the total number of adult flies emerging from pure cultures in days from the egg stage of the oldest larvae in each culture.

TABLE 3
Larval viabilities in mixed cultures

Age structure	A2 competition			A6 competition			F ₁ competition		
	+ / +	<i>e/e</i>	Both genotypes combined	+ / +	<i>e/e</i>	Both genotypes combined	+ / +	<i>e/e</i>	Both genotypes combined
+ / + = <i>e/e</i>									
Percent survival	90.50	74.75	82.62	21.12	9.87	15.50	61.62	24.62	43.12
mean number/vial	36.20 ± .51	29.90 ± .86	66.10 ± .86	8.45 ± 1.41	3.95 ± 1.04	12.40 ± 1.94	24.65 ± 1.45	9.85 ± 1.05	34.50 ± 2.09
+ / + > <i>e/e</i>									
Percent survival	71.12	68.87	70.00	39.87	36.75	38.43	80.25	19.87	50.06
mean number/vial	28.45 ± 1.84	27.55 ± 1.16	56.00 ± 2.70	15.95 ± 1.24	14.70 ± 1.23	30.65 ± 1.32	32.10 ± .84	7.95 ± 1.63	40.05 ± 1.91
+ / + < <i>e/e</i>									
Percent survival	71.87	69.50	70.68	68.25	73.87	71.06	56.00	34.12	45.06
mean number/vial	28.75 ± 1.52	27.80 ± 1.01	56.55 ± 2.14	27.30 ± 1.46	29.55 ± 1.15	56.85 ± 1.94	22.40 ± 1.25	13.65 ± 1.38	36.05 ± 2.06

somewhat shortened in the tests at unequal age structure although the bimodal distribution of developmental time at this age structure can nevertheless still be discerned. It is also of interest that in tests at equal age structure, the F_1 shows a longer developmental time than any of the other three strains and also shows a bimodal emergence pattern. The bimodal pattern may be caused by the fact that the F_1 was produced from reciprocal crosses between A2 and A6, and the progeny of each cross is subject to a maternal effect.

The results of the viability tests in mixed cultures are shown in Table 3. With the exception of A2 in equal age competition with ebony, the viabilities of all genotypes have significantly diminished compared to their viabilities in pure cultures at a density of 40 (Table 1) and this is probably caused by an increased density of competing larvae. It is also obvious that the viability of larvae in mixed cultures is affected by the kinds of genotypes competing as well as by their age structures. An analysis of variance of ebony viabilities in its nine different competing combinations (Table 4) shows significant effects caused by both of these factors. In addition, there is a significant effect caused by interaction between genotype and age structure so that in one instance, for example, the viability of ebony is increased when it is of equal age to wild type (A2) and in another instance it is decreased (A6). Generally, the presence of A2 enhances the viability of ebony compared to its average viability in these tests, whereas the presence of A6 and F_1 diminishes it. t -tests show that the F_1 maintains significantly higher viabilities than ebony in each competition, whereas the viabilities of A2 and A6 are significantly increased over ebony only in equal age competitions. It is interesting to note that although the F_1 is the most successful competitor to ebony, its average viability in these tests is less than that of A2. When the age of ebony larvae is increased, the survival of ebony is generally increased, or, at least, not diminished. However, in no instance is the viability of ebony significantly higher than the viability of its competing wild-type genotype.

These effects of genotype, age structure, and interaction can also be seen when the total numbers of flies emerging from each culture are scored. As shown in Table 5, an analysis of variance for this measurement again shows significant effects for each of the three sources of variation, with the effect of interaction now relatively increased. t -tests comparing the means of total population sizes in

TABLE 4
Analysis of variance for the viability of ebony in mixed cultures

Sources of variation	Sum of squares	df	Mean square	F
Genotype	10,106.01	2	5,053.005	179.18***
Age structure	2,711.51	2	1,355.755	48.08***
Genotype $\times$ age structure	4,301.69	4	1,075.422	38.14***
Subtotal	17,119.21			
Error	4,822.10	171	28.199	
Total	21,941.31	179		

*** $P < .001$

TABLE 5

Analysis of variance for the combined viabilities of both genotypes in mixed cultures

Sources of variation	Sum of squares	df	Mean square	F
Genotype	24,326.34	2	12,163.170	160.29***
Age structure	4,519.68	2	2,259.840	29.78***
Genotype $\times$ age structure	17,067.16	4	4,266.790	56.23***
Subtotal	45,913.18			
Error	12,975.55	171	75.880	
Total	58,888.73	179		

*** $P < .001$

mixed cultures to the population sizes observed in pure cultures (density of 80) show that there are significant improvements in the viabilities of the populations in A2 and A6 mixed cultures compared to A2 and A6 pure wild-type cultures when ebony has the same age structure as wild type (A2) or when ebony has a different age structure (A6). However, population sizes of F_1 mixed cultures show a significant deterioration of viability compared to those of pure F_1 cultures, no matter what the age structure. This might, therefore, suggest that population viability can be increased by heterogeneity when the population is relatively genetically homozygous (A2 and A6), but is decreased by heterogeneity when the population is already heterozygous (F_1). On the other hand, the fact that these viability changes are dependent upon the age structure of the competing ebony larvae in the case of the A2 and A6 populations indicates that genetic homozygosity alone is not sufficient to explain these results.

Average developmental times for the mixed culture genotypes are given in Table 6. As expected from previous considerations, tests at unequal age structure show an increase in average developmental time of approximately one day for the genotype with the younger larvae. Generally, average developmental time is significantly decreased with increasing viability for all genotypes ($r = -.54$, $P < .05$), although this is associated mainly with ebony larvae ($r = -.68$, $P < .05$) rather than with wild-type larvae ($r = -.39$, $P = .3$). Despite these negative correlations, poor viability is not necessarily always accompanied by long developmental times (e.g., A6 and its ebony competitor at equal age structure).

Emergence patterns of adults from each of the nine competitive conditions are shown in Figure 2. Although they are not too grossly dissimilar at unequal age

TABLE 6

Mean egg-to-adult developmental periods in days for each genotype in mixed cultures

Age structure	A2 competition		A6 competition		F_1 competition	
	A2	e/e	A6	e/e	F_1	e/e
+/+ = e/e	10.14 $\pm$.02	11.22 $\pm$.04	12.47 $\pm$.14	12.65 $\pm$.19	12.27 $\pm$.06	14.05 $\pm$.12
+/+ > e/e	12.19 $\pm$.06	13.02 $\pm$.05	12.71 $\pm$.11	13.74 $\pm$.08	12.30 $\pm$.05	14.54 $\pm$.13
+/+ < e/e	13.02 $\pm$.04	12.08 $\pm$.05	13.43 $\pm$.05	11.85 $\pm$.05	14.69 $\pm$.06	12.34 $\pm$.12

FIGURE 2.—Percentages of the total number of adult flies emerging from mixed cultures at the different indicated age structures. The days of emergence are counted from the egg stage of the oldest larvae in each culture.

structures, the patterns nevertheless vary; more overlapping between ebony and wild-type emergence patterns seems to occur for A2 than for ebony and the other two wild-type genotypes, especially the F₁. Also, the F₁ emergence patterns seem more normally distributed at unequal age structures than do those of the other two wild-type genotypes. On the other hand, in the three competitions at equal age structure, the emergence patterns are obviously different in each case, both for the wild-type genotypes and for ebony. At this age structure, A2 shows the most rapid and least variable emergence pattern of all genotypes and shows also the highest viability. A6, by contrast, has a bimodal pattern and very low viability, as does its ebony competitor.

DISCUSSION

The present experiment was designed to discover whether the early developmental stages of genotypes in competitive situations, adjusted by using larvae of different ages, would affect their relative fitnesses in single generation experiments. For this purpose, larvae from an ebony stock, and from three wild-type genotypes, A2, A6, and the F₁ between them, were tested both individually in "pure" cultures and in competition, ebony larvae × wild-type larvae, in "mixed" cultures. In accord with previous findings (CHIANG and HODSON 1950, Table 9; but see also BAKKER 1961, Table 1, and JONES and BARKER 1966, Figure 6), the

pure culture results show that an increase in density from 40 to 80 larvae per vial causes a decrease in percent viability as measured by the number of adults emerging. A change in the age structure of larvae at the 80 density, from all larvae of a genotype competing at equal ages to 40 larvae being one day older than the others, significantly affects all genotypes but the F_1 , causing either a significant increase (A2, A6) or decrease (ebony) in percent viability.

Of interest is the finding that, at the 80 density, the A6 wild-type genotype has even lower viability than the mutant genotype ebony, and that the highest viability is not that of the F_1 , as may have been expected from LEWONTIN's results (1955; see "random heterozygotes") and from those in competition experiments (DAWOOD and STRICKBERGER 1964, and below), but is instead found in ebony at equal age structure and A2 at unequal age structure. The striking results of the A6 genotype, in which the emergence pattern at equal age structure shows a marked lengthening for some individuals, indicate that the larvae of some genotypes may produce products that cause interference in pure cultures so that the development of a significant number of larvae to the adult stage is delayed.

The lengthening of *Drosophila* developmental time because of increased intra-strain competition can also be surmised from the studies of SOKOLOFF (1955, Table 8) in which the egg-to-adult interval generally increased for both *D. pseudoobscura* and *D. persimilis* at 24°C with increased larval densities in pure cultures. SOKOLOFF does not give information on emergence patterns but his standard errors indicate that the delay in eclosion at high densities is accompanied by an increase in variability of developmental period as well as by an increase in mortality (SOKOLOFF 1955, Table 3). This, however, is not an unalterable rule since the present A2 results at equal age structure show that a relative delay in the developmental period of a pure culture need not necessarily be accompanied by an increase in variance or even by a decrease in viability. In these comparisons, it is important to note that impaired viability cannot be simply explained through a physical shortage of food since, as shown by A2, for example, sufficient food exists in the present cultures to produce twice the number, and perhaps even twice the biomass, of emerging A6 adults. Thus, if we define interference as the inhibition of development of some individuals because of the presence of others (see LEWONTIN 1955; LEWONTIN and MATSUO 1963), there is little question that interference occurs in A6 cultures between individuals of the same genotype.

In mixed cultures, interference can be observed between different genotypes in the F_1 and A6 competitions. The F_1 causes a marked reduction in the frequency of ebony no matter what the larval age structure used to initiate the culture. That this is not merely competition for resources present in short supply can be surmised from the fact that the combined numbers of flies emerging in F_1 -ebony mixed cultures are generally significantly less than the numbers produced by these genotypes in pure cultures at densities of 80. A6 and ebony at equal age structures show the greatest mutual interference between genotypes recorded in this experiment, yielding a total of only 248 adults out of 1600 larvae. Only at unequal age structure competitions are the survival values of ebony and A6 genotypes improved, reaching a maximum when ebony larvae are older than A6.

These changes in survival signify marked viability interactions between larvae, and are made especially obvious when comparing the F_1 and A6 results to those of A2. If we define facilitation as the enhancement of the viability of some individuals because of the presence of others (see LEWONTIN 1955; LEWONTIN and MATSUO 1963; BEARDMORE 1963), it is obvious that A2 acts as a general "facilitator," maintaining not only its own viability but improving that of its ebony competitor. For example, the equal age structure competition with ebony in which the greatest reduction in total larval survival occurs for the F_1 and A6 competitions, yields the greatest total survival when using A2. We can also note that, on the average, a greater total number of flies emerges at densities of 80 when ebony and A2 larvae are in the same culture (1191) than when they are separate (851, 981).

The exact nature of interference and facilitation in the present experiments is difficult to discern. Generally, for the "interfering" genotypes competing with ebony (A6 and F_1) an improvement in viability accrues to the genotype of the older larvae in the culture. This view is supported by the results of BAKKER (1961, p. 261) who showed that a mere increase of 3 hours in the age of *Bar* mutant larvae of *D. melanogaster* compared to the age of their wild-type competitors led to a marked improvement in viability of the *Bar* genotype; and when this relative increase in age was raised to 12 hours, wild type was completely eliminated. Although the results of the present experiment are not as dramatic as those of BAKKER (perhaps because of the smaller numbers of competing larvae per culture used in this experiment) they do indicate that younger larvae are more sensitive to interference, a view also indicated by other *Drosophila* workers (L'HÉRITIER and TEISSIER 1934; SANG 1949). Since there is no evidence that interference takes the direct form of either physical attack or cannibalization in *Drosophila*, it has been variously suggested (BODENHEIMER 1938; CHIANG and HODSON 1950; LEWONTIN 1955; BAKKER 1961; and others) that interference occurs through metabolic substances secreted by larvae into the food medium. Evidence that such inhibitory substances are secreted by the genotypes in the present experiment is given in an accompanying paper (DAWOOD and STRICKBERGER 1969; see also WEISBROT 1966). The same explanation can also be used to explain facilitation; that is, some facilitation effects can occur not merely because of the absence of interference, but because of metabolic secretions that improve the viability of coexisting larvae. The likelihood that such facilitating substances are produced by some combinations of the presently used genotypes is also presented in the accompanying paper (DAWOOD and STRICKBERGER 1969).

One question that can be asked of the present data is whether they reflect upon the relative fitnesses of ebony and wild-type genotypes in other single generation experiments. Despite the various interactions observed in mixed cultures of the present experiment, it is interesting to note that the F_1 is the most consistent superior competitor to ebony. This is a result that supports the conclusion reached in the previous experiment (DAWOOD and STRICKBERGER 1964) that the degree of heterozygosity of a genotype is directly related to its fitness (see also POLIVANOV 1964). However, as pointed out by LEWONTIN and MATSUO (1963) and STRICK-

BERGER (1963), population fitness measured in biomass or numbers is not always correlated with genotypic fitness measured in competitive frequency, and the present experiments demonstrate clearly that a genotype which appears to have relatively poor competitive ability over ebony in mixed cultures, e.g., A2, may nevertheless have a facilitating effect on the fitness of the entire population.

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SUMMARY

Two *Drosophila melanogaster* wild-type stocks, the F_1 between them, and an ebony stock were tested for viability at densities of 40 and 80 larvae per vial in pure cultures and at densities of 80 larvae per vial in ebony-wild type mixed cultures. At the higher density, the age structures of the larvae were controlled so that competition occurred between all larvae at equal ages or between 40 larvae one day older than the 40 others. The results showed that viability generally decreased with increasing density, and that at the higher density, viability depended upon the genotype, the age structure of competing larvae, and upon interaction between these two factors. The F_1 in mixed cultures consistently showed the highest competitive ability over ebony, whereas one of the other wild-type genotypes had a facilitating effect on ebony viability, and the remaining wild-type genotype generally had an inhibitory effect, both on ebony and itself. There was no correlation between genotypic success over ebony in mixed cultures and population fitness measured by the total number of flies emerging per mixed culture. Generally, a lengthening of average egg-to-adult developmental time accompanied decreasing viability, although some exceptions to this rule existed. It is suggested that genotypic viabilities in these experiments are affected by substances secreted into the food medium by competing larvae.

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