# THE EFFECT OF LARVAL INTERACTION ON VIABILITY IN *DROSOPHILA MELANOGASTER*. III. EFFECTS OF BIOTIC RESIDUES

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CHANGES in the viability of Drosophila genotypes have been shown to occur because of changes in larval density (SANG 1949; CHIANG and HODSON 1950; BIRCH 1955; MOREE and KING 1961; and others), changes in the presence of larvae of other genotypes (LEWONTIN 1955; BAKKER 1961; LEWONTIN and MATSUO 1963; DAWOOD and STRICKBERGER 1969; and others), and changes in the degree of heterozygosity of larvae of other genotypes (DAWOOD and STRICKBERGER 1964). Many of these experiments indicate that a significant portion of the observed viability effects are probably mediated through substances secreted by larvae into the common culture medium. To further investigate these relationships the present experiments were designed to measure the viability of genotypes whose larvae are placed in cultures containing the biotic residues left there by older but no longer living larvae (see also WEISBROT 1966).

## MATERIALS AND METHODS

The four genotypes of *Drosophila melanogaster* used in the present experiments are the same used in previous investigations (DAWOOD and STRICKBERGER 1969) and consist of two laboratory wild-type stocks, A2 and A6, the $F_1$ hybrid between them, and an ebony mutant stock ($e^{11}$). Freshly hatched larvae, uniformly 4–6 hrs old, were collected by the methods previously described (*ibid.*) and used both to condition the culture medium and for viability tests. For conditioning of the medium, 40 larvae of a particular genotype were placed in 25 × 95 mm shell vials containing 5cc of KALMUS sucrose-agar medium seeded with 5 mg yeast in 0.1 cc water. After a two-day period of conditioning by these live larvae, the vials were placed in a confined chamber over dry ice for approximately 15 min. (We are indebted to PROFESSOR E. R. DEMPSTER for suggesting this technique.) This process freezes the food medium and causes the death of all larvae within these cultures. The vials were then permitted to thaw and, upon reaching room temperature (25°C), 80 larvae of the genotype to be tested, 4–6 hrs old, were added to the conditioned food medium in the vial. Each of the four genotypes was tested in cultures whose media had been conditioned by its own larvae and in separate cultures conditioned by larvae of each of the other three genotypes. In addition, control studies were made of the viability of each genotype by placing larvae in cultures containing food medium that had been frozen for 15 min but which had not been conditioned by previous larvae. Each test was performed at 25°C in 20 replicate vials. Newly emerging flies were counted daily to obtain mean developmental periods and emergence patterns for each test.

## RESULTS

The viabilities of the four genotypes under each of the conditions tested are shown in Table 1. Statistical *t* tests for A6 and $F_1$ viabilities show that the results

TABLE 1

*Viability of genotypes in each of the differently conditioned media*

| Medium conditioned by: | | Genotype A2 | A6 | $F_1$ | ebony | Average percent survival for all genotypes |
|---|---|---|---|---|---|---|
| Control | Percent survival | 54.19 | 26.00 | 60.44 | 58.69 | 49.82 |
| | mean/vial | 43.35±1.94 | 20.80± .91 | 48.35±2.64 | 46.95±2.95 | |
| A2 | Percent survival | 60.19 | 79.13 | 66.31 | 67.75 | 68.34 |
| | mean/vial | 48.15±2.53 | 63.30±1.04 | 53.05±2.67 | 54.20±3.40 | |
| A6 | Percent survival | 36.63 | 34.94 | 51.13 | 5.25 | 31.98 |
| | mean/vial | 29.30±1.39 | 27.95±2.74 | 40.90±1.54 | 4.20±1.53 | |
| $F_1$ | Percent survival | 61.94 | 38.38 | 86.94 | 76.06 | 66.57 |
| | mean/vial | 49.55±2.23 | 30.70±1.63 | 69.55± .64 | 63.25±1.21 | |
| ebony | Percent survival | 34.06 | 3.19 | 59.94 | 60.94 | 39.53 |
| | mean/vial | 27.25±2.44 | 2.55± .42 | 47.95±1.95 | 48.75±2.33 | |
| Average percent survival on all media | | 49.40 | 36.32 | 64.95 | 54.33 | |

of the control experiments (frozen but unconditioned medium) are directly comparable ($P > .8$) to the results obtained previously for pure cultures at 80 equal ages (Dawood and Strickberger 1969, Table 1). The A2 and ebony control viabilities, however, are approximately 11% lower than the previous results. For ebony, this reduction is almost significant at the .05 level, whereas for A2 the difference is highly significant ($P < .001$). Such viability changes may, of course, arise from genetic changes (e.g., contamination), although such an explanation is not too likely in view of the consistency of the facilitating effect shown by A2 in the larval conditioning experiments, a result that is fully in accord with its previous pattern of interaction. In addition, contamination of the ebony stock, had it occurred, would most likely have been caused by wild-type flies and resulted in an observable change in phenotype. Since such contaminations were not observed, it seems more likely that the changes are environmentally caused and that freezing of the medium may in some way reduce the viabilities of A2 and ebony but has little or no effect on A6 and $F_1$.

In the larval conditioning experiments, the facilitating effect of A2 on the food medium is obvious and produced the highest average survival rate. Compared to the control experiments, the viabilities of all genotypes under A2 conditioning increased, although only in the case of A6 is this increase significant ($P < .001$). Interestingly, the facilitating effect of A2 on other genotypes does not reflect its own relative viability. With the exception of A6 conditioning (where the viability of ebony is significantly lower), A2 viability is lower than that of $F_1$ and ebony, both on conditioned medium and in controls. In many instances ($F_1$ conditioning, ebony conditioning, the $F_1$ under A6 conditioning) this comparative reduction in A2 viability is highly significant ($P < .001$).

The second genotype with facilitating effects in this experiment is the $F_1$ as indicated by the significant increase in viability of all genotypes compared to the

controls when placed on $F_1$-conditioned medium. Also, for three of the genotypes (A2, $F_1$, ebony) the results on $F_1$-conditioned medium are higher than in any of the other tests, and this relative increase is significantly greater than all other values for two of these genotypes ($F_1$, ebony). The approximate 87% viability of the $F_1$ on $F_1$-conditioned medium is the largest recorded in this experiment and exceeds all others ($P < .001$). This high value helps to give the $F_1$ the highest average survival rate of all genotypes (64.95%), although this is also partly caused by the fact that $F_1$ viability on A6-conditioned medium is significantly higher than all others.

The interference effects observed previously for A6 (DAWOOD and STRICKBERGER 1969) continue in the present experiments on A6-conditioned medium, resulting in the lowest average survival per genotype (31.98%). The ebony genotype suffers relatively most because of this interference, while the viability of A6 itself appears relatively increased compared to the controls. Interestingly, the ebony genotype also produces significant interference effects of its own when used to condition the medium and causes the lowest viabilities observed for the A2 and A6 genotypes. The A6 viability on ebony-conditioned medium is lower than that of any other genotype, with significance in every comparison ($P < .001$) except for the viability of ebony on A6-conditioned medium. This mutual interference effect between A6 and ebony seems to parallel the effect noted previously for mixed cultures when A6 and ebony larvae were of equal ages (*ibid.* Table 3).

Mean egg-to-adult developmental times shown in Table 2 are, on the whole, negatively correlated with viabilities ($r = -.48$, $P < .05$), although exceptions exist. (Note, for example, the general increase in developmental time for genotypes raised on A2 medium compared to control medium despite the facilitating effect of A2 medium.) Also of interest is the relative constancy of the developmental period for all genotypes on ebony medium at approximately 12.5 days even in the instance where viability has been drastically reduced (A6 genotype). The interference effect of ebony medium therefore seems to act somewhat differently from the interference effect caused by A6 medium in which developmental time of the ebony genotype is considerably increased. Another factor that also indicates differences in type of interference effects between the two genotypes is the visual observation that many of the ebony larvae cultured on A6 medium

TABLE 2

*Mean egg-to-adult developmental period in days for the four genotypes in differently conditioned media*

| Medium conditioned by: | Genotype | | | |
|---|---|---|---|---|
| | A2 | A6 | $F_1$ | ebony |
| Control | 10.64±.04 | 11.35±.04 | 12.21±.02 | 11.52±.03 |
| A2 | 12.24±.04 | 11.59±.02 | 13.32±.06 | 12.22±.03 |
| A6 | 11.74±.03 | 13.20±.06 | 12.14±.05 | 14.40±.25 |
| $F_1$ | 11.47±.04 | 11.79±.06 | 11.06±.02 | 11.19±.02 |
| ebony | 12.34±.05 | 12.53±.24 | 12.52±.05 | 12.55±.03 |

moved quite slowly over the food surface and pupation seemed considerably delayed in those that survived, whereas most A6 larvae cultured on ebony medium left the food surface within a few hours after transfer to the food medium and then wandered on the walls of the vial until they died. Emergence patterns, as shown in Figure 1, also indicate this difference and show, in addition, a multipeak emergence pattern for the ebony genotype cultured on A6 medium. This may indicate either heterogeneity in the ebony stock in respect to its ability to complete development on A6 medium, or, since there is very little penetration of the food medium, only few ebony larvae at a time are able to obtain sufficient nutrients from the surface-growing yeast to develop successfully.

On the other hand, the interference effect of the ebony-conditioned medium on A6, as well as on other genotypes, results in a more unimodal emergence pattern. Note also that the unimodal pattern is markedly exaggerated for genotypes raised on $F_1$-conditioned medium, especially for the $F_1$ and ebony genotypes in which the facilitating effect of $F_1$ medium is greatest. The $A_2$ medium, by contrast, appears to produce its facilitating effect in a somewhat different manner since emergence pattern peaks on this medium are considerably more flattened, especially in the case of the $F_1$.

## DISCUSSION

In 1950, CHIANG and HODSON pointed out that larval viability in *Drosophila melanogaster* was affected by the density of larvae in their experiments although

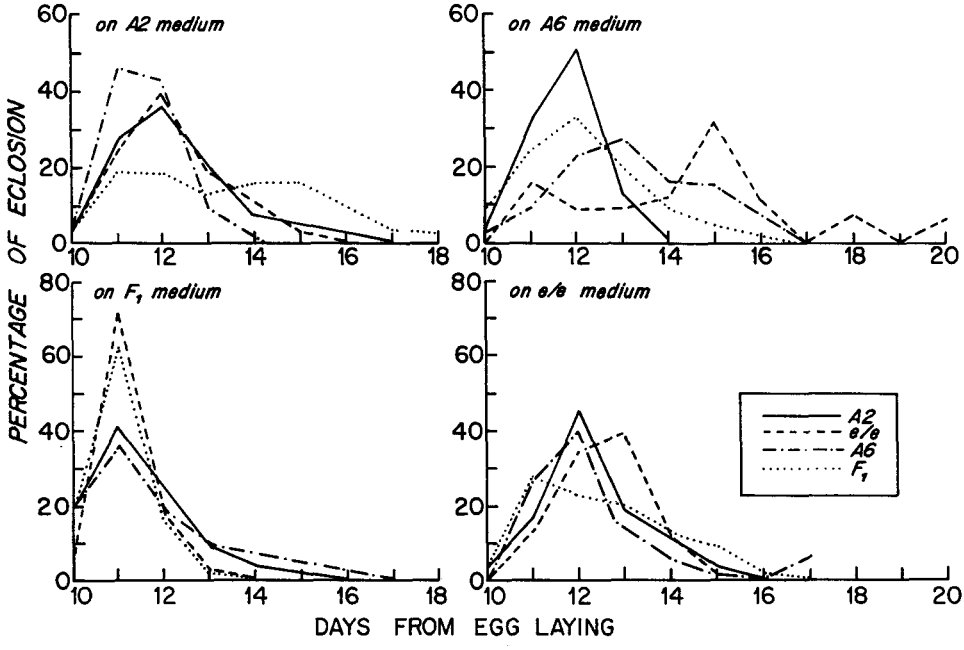


FIGURE 1.—Percentages of the total number of adult flies emerging on differently conditioned media.

each larva was provided with the same amount of food at all of the various densities used. They offered the possible explanation that this effect was caused by waste products excreted by the larvae. Similar views were presented by BODENHEIMER (1938), LEWONTIN (1955), BAKKER (1961), and others. To demonstrate that this effect can be caused by metabolic products and not merely by direct physical contact between living Drosophila larvae has demanded the development of a technique which permits the viability of larvae to be measured on medium containing products deposited by other larvae that are no longer present or active. The present experiments, utilizing food medium conditioned by larvae killed after two days by freezing, show conclusively that the metabolic products of such larvae may have significant viability effects, both in terms of interference and facilitation, on later larvae placed on this same medium. WEISBROT (1966), who adopted essential features of this technique, showed also viability effects in terms of interference between genotypes, but did not observe facilitation between the present genotypes.

It is, of course, possible in these experiments that the dead larvae themselves are primarily responsible for the observed viability effects, either through their physical presence or through their decomposition products. However, mere physical presence of dead larvae seems to be an insufficient explanation since the same number of dead larvae (40) are present in each test and would hardly be expected to give such divergent results. Since it is obvious that it is the genotype of the dead larvae that is important, one may argue that special decomposition products peculiar to each genotype are produced by the dead larvae. One factor that indicates a small role, if any, for such decomposition products is the observation that viability interactions between these genotypes have occurred even in the absence of killed larvae, as reported previously (DAWOOD and STRICKBERGER 1969). Also, as observed presently for A6 larvae on ebony medium, some of the interactions occur within a short time after the placement of larvae on the conditioned medium before much decomposition of the dead larvae could have occurred. Furthermore, WEISBROT (1966) tested the effect of dead larvae which did not have the opportunity to condition the food medium by placing 3-day-old larvae on unconditioned medium and then killing them immediately afterwards. He found that the physical presence of these dead larvae had no viability effect on later larvae placed on this medium.

In comparing the present results with those obtained previously for interactions between live larvae of the same four genotypes (DAWOOD and STRICKBERGER 1969) a few notable features stand out. One finding is that the general facilitation effect of the A2 genotype and the interference effect of A6 is present in both experiments and indicates some measure of predictability for these genotypes for the two techniques. The effect of A2-conditioning on the food medium is, in fact, sufficiently great in the case of A6 larvae that it triples their average viability as determined from other media. A2 can obviously supply what A6 lacks, although the reverse is not true.

Consistency between the present and previous results, however, breaks down for the $F_1$ and ebony genotypes. The $F_1$, which previously appeared to have an

interference effect on ebony, now has a marked facilitating effect when ebony is raised on $F_1$-conditioned medium. The ebony genotype, formerly showing a facilitating effect on A6 when ebony larvae were older, now has a marked inhibitory effect on A6 larvae, reducing their viability to the lowest observed in these experiments. This interference effect of ebony-conditioned medium extends also to A2, a result that could not have been predicted from the relatively high viabilities of A2 that were observed previously in the presence of older ebony larvae. It is, therefore, clear that experiments using the biotic residues of genotypes need not necessarily reflect results obtained from live competition.

Reasons for this disparity may come from the fact that larvae are continually metabolizing the medium and the products of this metabolism probably change with time. The fact that conditioning of the medium by two-day-old ebony larvae markedly reduced the viability of A6, but that the continued presence of older ebony larvae enhances the viability of A6 shows clearly that different substances are probably involved. Also, WEISBROT (1966), using three of the genotypes in the present experiment, performed his tests by allowing larvae to condition the medium for three days instead of only two and observed an interference effect of A2-conditioned medium rather than a facilitating effect. In accord with our explanation, this may have been caused by a change in the kind of product secreted into the medium by A2 larvae on the third day, although it is also important to note that WEISBROT used 40 larvae to measure viability rather than the 80 used presently.

One important indication that different kinds of interactions may be involved both in interference and in facilitation can be found in the developmental times and emergence patterns of the present experiment. For example, interference effects caused by ebony-conditioned medium on the A6 genotype do not appreciably lengthen developmental time as much as the interference effects caused by A6-conditioned medium on the ebony genotype, although the viabilities are approximately the same in both cases. The reverse holds true for the interference effect noted on larvae of the A2 genotype; their developmental time is increased on ebony-conditioned medium and, by comparison, significantly decreased on A6-conditioned medium, although their viability is reduced in both cases. Differences in the kinds of facilitation effects produced by A2 and $F_1$ are also apparent; the A2-conditioned medium improves genotypic viability by somewhat lengthening development time and flattening the emergence patterns (Figure 1) whereas the $F_1$-conditioned medium improves viability by reducing the developmental period and causing sharply peaked emergence patterns. The observation that $F_1$-conditioned medium has its greatest facilitating effect on the $F_1$ genotype may, at first, seem to explain observations of heterozygote superiority. Nevertheless, it should be noted that the $F_1$-conditioned medium also significantly improves the viability of ebony, a genotype which usually does poorly in competitive cultures (e.g., (DAWOOD and STRICKBERGER 1964).

The likelihood that both interference and facilitation may each be caused by different types of interaction, combined with probable differences in the types and amounts of residues produced by larvae as they mature, may therefore account

for the lack of predictability between past experiments done with live larvae and the present experiments done with conditioned medium. Variability of these factors may also account for the differences observed when the numbers and age structures of competing larvae are modified. It, therefore, seems clear that many complex population phenomena that concern viability and genotypic success among Drosophila larvae are mediated through substances secreted into the food medium.

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## SUMMARY

The viabilities of two *Drosophila melanogaster* wild-type stocks (A2, A6), the $F_1$ between them, and an ebony stock, were each tested on food medium previously conditioned by allowing larvae of each of these genotypes to live on separate samples of medium for a two-day period. Tests performed on unconditioned medium were used as controls. Compared to the controls, two of the genotypes (A2, $F_1$) conditioned the medium so that the viabilities of larvae placed upon this medium were significantly improved (facilitation). The two other genotypes (A6, ebony) conditioned the medium so that the results observed were generally lower than those of the controls (interference). In general, considerable interaction was observed and the viability of any particular genotype varied in different conditioned media. Developmental egg-to-adult periods were, on the whole, negatively correlated with viability, although exceptions existed. Data on developmental period and on daily emergence patterns indicated that both interference and facilitation could each occur as a result of more than one type of interaction. The causes responsible for these interactions apparently involve diffusible metabolic products produced by larvae in the food medium and their quantity, or quality, or both, are most likely dependent on the numbers and age structures of the larvae.

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