GENOTYPIC INTERACTIONS AMONG COMPETING STRAINS AND SPECIES OF DROSOPHILA

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EVIDENCE is accumulating to support the observation that the fitness of a particular genotype in a given environment may be improved or impaired by the presence in that same environment of conspecific carriers of other genotypes. This has been shown in *Drosophila pseudoobscura* by LEVENE, PAVLOVSKY and DOBZHANSKY (1954), and BEARDMORE (1963); in *D. melanogaster* by LEWONTIN (1955); in *D. busckii* by LEWONTIN and MATSUO (1963); in *Tribolium* by PARK (1936), NEYMAN, PARK and SCOTT (1958), SOKAL and KARTEN (1963) and KARTEN (1965); in rice by ROY (1960); and in barley by GUSTAFFSON (1957). For additional references, see ALLEE, EMERSON, PARK, PARK, and SCHMIDT (1949) and SLOBODKIN (1958). POLIVANOV (1964) and DAWOOD and STRICKBERGER (1964) found that in *D. melanogaster* the frequency of the mutant gene ebony is decreased more rapidly in competition with a heterozygous than with an inbred wild-type strain. Furthermore, the latter authors (unpublished) have shown that under conditions of crowding, (160 larvae per 5 ml of medium), the survival rate of ebony larvae is lower when grown on media containing either the larvae or biotic residue of a Samarkand strain (A6) than with an inbred strain (A2). The following experiment was undertaken in an attempt to clarify the effect of media conditioned by one genotype upon the fitness of competing genotypes.

MATERIALS AND METHODS

Four strains of *D. melanogaster* and one strain of *D. pseudoobscura* were employed in the present work. Of the *D. melanogaster* strains, A2 is a California strain made coisogenic in 1948, and maintained since that time in DR. C. STERN's laboratory at the University of California in Berkeley, while A6 designates a mass mated Samarkand strain. The symbol, *ee* refers to a homozygous ebony mutant strain, and *bw;e* to a homozygous double mutant (brown-eyed, ebony body color); the latter ebony is of a different origin than *ee*. *D. pseudoobscura*, P1 is taken from a population cage #173, now maintained in DR. M. STRICKBERGER's laboratory at St. Louis University, and is polymorphic for the Arrowhead and Chiricahua third-chromosome gene arrangements.

Virgin females and mature males were placed together in an empty half-pint bottle, inverted over a plastic cap containing cream of wheat-molasses-agar medium lightly yeasted with a 10% suspension of baker's yeast. The cap is replaced every 24 hours with a fresh one and the removed cap stored at 25°C for an additional 24 hours. Thus, larvae no more than 24 hours old are col-

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lected by picking them from the surface of the stored caps with a fine needle, and transferring them to vials containing 5 ml of KALMUS medium (KALMUS 1943) seeded with 3 drops of a 10% live yeast suspension. Forty larvae are placed in each vial, with about ten replicate vials run for each genotype both under control and under experimental conditions. Unless otherwise specified, the larvae are allowed to grow on the medium for three days in order to condition it. The larvae are then killed by placing the vials on dry ice for 1 hr, and following a 1 hr period of thawing at room temperature, a second group of 40 larvae is added to each vial. This group is either of the same strain as those conditioning the medium (homotypic) or of a different strain (heterotypic). The survival rate is measured by the number of emerging adults and is reported as the mean numbers of adults (survivors) per vial in a series of replicates.

To determine whether the competitive interaction is mediated by the live yeast or reflects a direct effect between the larvae, SANG's defined medium (SANG 1956) without live yeast was used for some experiments in place of the Kalmus medium. Eggs collected from A6 and *ee* were suspended in a 3% sodium hypochlorite solution for 1 hr to dechorionate them and then were washed in distilled water. The eggs were then transferred to a 2% solution of Centrimide (I.C.I. Industries), a fungicide and bactericide, for about 1 hr, and then resuspended in distilled water. After the second washing, the eggs were transferred in groups of 40 to vials containing the sterile medium, using sterile technique. After seven days at 25°C, the vials were frozen on dry ice. After thawing of the sterile A6 and *ee* conditioned media, both washed *ee* and A6 eggs were added in separate experiments to both types of media.

RESULTS

To determine the viability of the strains on the Kalmus medium, ten replicates each of 40 larvae per vial are run on pure (unconditioned) medium. The larvae used are no more than 24 hours old. The results are reported in Table 1, and it can be seen that both A6 and *ee* larvae show a relatively high survival rate on

TABLE 1
Average survival of larvae on various media

Medium	Larvae added				
	A2	A6	<i>ee</i>	<i>bw;e</i>	P1
Nonconditioned	13.6 ± 1.6 (10)*	32.2 ± 0.9 (10)	32.3 ± 1.4 (10)	24.6 ± 1.4 (10)	15.0 ± 2.9 (10)
Nonconditioned (pre-frozen)	13.8 ± 1.2 (10)	27.0 ± 1.3 (10)	28.2 ± 1.7 (9)	23.4 ± 1.7 (9)	2.3 ± 0.8 (9)
Conditioned					
Dead A2	17.5 ± 1.1 (18)	17.6 ± 1.4 (26)	12.0 ± 2.1 (11)		0.6 ± 0.2 (20)
Dead A6	15.3 ± 1.6 (22)	25.4 ± 2.3 (18)	11.0 ± 1.4 (40)	6.0 ± 1.4 (20)	2.6 ± 0.6 (16)
Dead <i>ee</i>	16.1 ± 2.8 (15)	20.3 ± 1.6 (18)	24.0 ± 2.4 (11)	11.0 ± 3.1 (10)	
Dead <i>bw;e</i>		25.7 ± 2.3 (14)	19.4 ± 1.0 (7)	16.2 ± 4.2 (4)	
Dead P1	22.3 ± 1.2 (40)	28.2 ± 1.6 (40)			3.7 ± 0.6 (17)

* Number of replicates is shown in parentheses.

Survival per vial is shown, starting with 40 24-hour larvae of one strain grown on 5 ml of Kalmus medium that had not been previously conditioned; that had been frozen, but not conditioned; and had been conditioned for 3 days by growth of larvae of the same (homotypic) or of a different (heterotypic) strain. The survival rate is measured by the number of emerging adults.

the Kalmus medium, compared with the poor showing of A2 and P1. These results are compared with studies when larvae are added to nonconditioned, but previously frozen medium, indicating that the freezing process has little or no effect on *bw;e* or A2, but does reduce larval survival of the other strains. Table 1 further compares the effect of nonconditioned versus homotypically conditioned medium on larval survival of the various strains employed in this study. The data indicate that A2 larvae show a consistent, though nonsignificant, increase in survival on conditioned as compared with nonconditioned medium. This is contrary to the results obtained for all other strains tested which show a consistent, though nonsignificant, decrease in survival on homotypically conditioned medium compared with nonconditioned medium cultures. DAWOOD and STRICKBERGER (1964) found that under more crowded conditions (80 larvae added to each vial of homotypically conditioned medium), A2 larvae show as high a rate of survival as either A6 or *ee*. P1 larvae show a significantly lower rate of survival on homotypically-conditioned than on pure medium.

Finally, Table 1 compares the survival rates of larvae grown on homo- and heterotypically conditioned medium. All strains tested other than A2 show a decrease in survival when grown on A2-conditioned medium when compared with homotypically conditioned media. For example, A6 larvae show an average survival rate of 25.4 ± 2.3 larvae per vial in homotypic cultures and 17.6 ± 1.4 on A2 conditioned medium, a significant decrease at the 5% level. Similarly, *ee* and P1 larvae have their survival rate significantly reduced from 24.0 ± 2.4 to 12.0 ± 2.1 larvae per vial for the former and 3.7 ± 0.6 to 0.6 ± 0.2 larvae per vial for the latter. DAWOOD and STRICKBERGER (1964) indicate that at higher densities of larvae per ml of medium, the inhibitory effect of A2-conditioned medium is ameliorated. The survival rate of *bw;e* larvae tested only on A6-conditioned medium is similarly suppressed to 6.0 ± 1.4 larvae per replicate compared with 16.2 ± 4.0 larvae in homotypic cultures. The data for the latter are not conclusive because of the low numbers of replicates and high variance.

To demonstrate that the effect is caused by the active larvae rather than by their mere presence, *ee* and A6 larvae are allowed to grow in separate collecting caps for three days before being transferred to Kalmus medium. They are frozen immediately, and are, therefore, of the same age as the frozen larvae in the conditioned medium. Under these conditions, the inhibitory effect on larval survival (previously observed when *ee* and *bw;e* larvae were grown on A6 conditioned medium) is no longer found. As shown in Table 2, the average survival per replicate culture is 32.0 ± 1.5 larvae for *ee* and 33.5 ± 3.0 for *bw;e* larvae on media in which the A6 larvae grow before they are killed. This observation therefore indicates that some agent (or agents) in the larval metabolic wastes cause the differential survival of competing larval genotypes.

In order to determine whether there is a particular stage especially sensitive to larval produced metabolites, eggs, 48- and 72-hour old larvae of both *ee* and A6 strains were grown in homo- and heterotypically conditioned medium for these experiments. The medium was conditioned for 48 rather than 72 hours used in earlier experiments. As shown in Table 3, the larvae generally show a

TABLE 2

Average larval survival in nonconditioned media containing dead larvae

Strains added at 3 days	Nonconditioned media containing dead larvae of the following strains	Number of replicates	Average survival per vial
<i>ee</i>	A6	9	32.0 ± 1.5
A6	<i>ee</i>	7	31.5 ± 2.4
A6	<i>bw;e</i>	8	29.7 ± 2.2
<i>bw;e</i>	A6	4	33.5 ± 3.0

Forty larvae of one strain are put on Kalmus medium 3 days *after* hatching so that they are killed at the same age as those under experimental conditions, except that they do not metabolize the medium.

higher survival rate except for *ee* eggs grown on A6 conditioned medium. Under these conditions, a slight but significant decrease in the survival rate of ebony larvae is observed when compared with ebony larvae grown on homotypically conditioned medium. The higher fitness for the stages tested is probably a consequence of the present reduction in length of the media-conditioning causing a reduction in the amounts of accumulated, detrimental waste products. An incidental outcome of this experiment is that *ee* eggs have a lower hatchability than their wild-type counterparts in both homo- and heterotypically conditioned media. (27.2 ± 1.4 and 26.9 ± 1.0 larvae survive for *ee* eggs compared with average survival for A6 eggs of 30.5 ± 0.9 and 29.9 ± 1.3). A comparison of the difference between the combined data gives a $t = 2.04$, significant at the 5% level.

To determine whether the larvae of different strains differentially affect the growth rate of the yeast, liquid Kalmus medium (without agar) was used to grow a yeast suspension, whose generation time was determined by periodical optical density reading. Of the six simultaneous cultures run for this experiment two represented control replicates, two had A6 and two had A2 larvae (40 larvae per 10 ml of suspension) added. These experiments showed a yeast generation time for the controls of $7\frac{1}{2}$ hours, compared with $3\frac{1}{2}$ hours for the yeast in the larvae-containing tubes, as measured by optical density. Figure 1 indicates these relative growth rates of the yeast. It is obvious that the Kalmus medium is un-

TABLE 3

Average larval survival in cultures begun with different stages of development

Strain added	Stage added	A6 conditioned medium		<i>ee</i> conditioned medium	
		Number of replicates	Average per vial survival	Number of replicates	Average per vial survival
A6	eggs	15	30.5 ± 0.9	15	29.9 ± 1.3
	48-hr larvae	19	29.5 ± 0.9	18	31.2 ± 0.9
	72-hr larvae	7	34.3 ± 1.6	7	32.7 ± 1.6
<i>ee</i>	eggs	20	27.2 ± 1.3	18	26.9 ± 1.0
	48-hr larvae	15	26.4 ± 1.6	13	31.7 ± 1.6
	72 hr larvae	17	26.9 ± 1.6	12	30.9 ± 0.9

Forty larvae of one strain are allowed to grow on 5 ml of Kalmus medium for 48 hours. They are then killed by freezing on dry ice. After the medium thaws, 40 eggs, or 48- or 72-hour-old larvae of the same or a different strain are added and their viability determined by the number of emerging adults.

FIGURE 1.—Growth rate of yeast with and without A6 and A2 larvae.

favorable for yeast growth but that the addition of larvae provides growth substances lacking in the medium. Since neither A2 nor A6 caused differences in yeast growth rate, this would indicate that the inhibitory effect on larval survival rates is probably independent of the yeast. Furthermore, when a defined medium (SANG 1956) is used and the entire experiment performed in the absence of yeast under aseptic conditions, the inhibitory effect of A6 conditioning on *ee* larval survival rates is still found (see Table 4). In this particular experiment, eggs are used instead of larvae because they could more easily be washed free of contaminating fungi and bacteria.

TABLE 4

Average larval survival on defined media without live yeast, frozen after 5 to 7 days of growth

Larvae added after freezing	Larvae added before freezing			
	A6		<i>ee</i>	
	Number of replicates	Average survival per replicate	Number of replicates	Average survival per replicate
A6	10	30.8±1.3	5	35.0±0.1
<i>ee</i>	5	16.6±2.4	4	29.0±3.3

Larvae of either the ebony or A6 strain were added to SANG's defined medium under sterile conditions in groups of 40 per vial and after 5 to 7 days of growth, the vials were frozen. Forty larvae of the same (control) or the opposite strain were then added to each vial.

DISCUSSION

POLIVANOV (1964) and DAWOOD and STRICKBERGER (1964 and unpublished results) have shown that, under competitive conditions, the ebony gene frequency is differentially reduced depending on the genotype of the competitors. The latter authors have further shown that the interaction among competing genotypes probably takes place at the larval stage, and is, in some way, influenced by the respective biotic residues. This suggests that in some instances, fluctuations in gene frequency may be due to the modification of the medium by metabolic products that act either by complementing the nutritional requirements of a particular genotype or by inhibiting the development of the competing genotypes, or through both mechanisms.

When A2 larvae condition the media, all other strains tested show a reduced rate of survival compared with their survival rates on homotypically conditioned medium. DAWOOD and STRICKBERGER (unpublished) showed that with greater numbers of A2 conditioning larvae per ml of medium, the rates of survival of competitive genotypes increased as did the numbers of A2 itself in homotypically conditioned cultures. Apparently, the inhibitory effect of A2-conditioned medium noted in the present experiments is due to a deficiency of the Kalmus medium that is not properly supplemented by A2 larvae unless such larvae are present in larger numbers. Furthermore, different strains or species may vary in their ability to supply this deficiency, as shown by the fact that *D. pseudoobscura* produces an increase in A2 survival that is significantly greater than that observed for A2 in pure cultures.

Unlike the A2-conditioned medium, A6-conditioned medium only reduces the survival rate of *ee* and *bw;e* among the strains tested. In addition, increased numbers of A6 conditioning larvae (DAWOOD and STRICKBERGER, unpublished) increases this inhibitory effect upon the survivorship of ebony larvae, suggesting that such larvae are sensitive to some product of the A6 larvae. In further contrast to the A2 effect, the A6 effect appears to be specific.

That these interactions are controlled by metabolic end products, and not by the presence of the larvae themselves was demonstrated by the results previously presented in Table 2. When grown on media in which dead larvae are present, but metabolic products are absent, the reduced rate of *ee* and *bw;e* survival on media conditioned by A6 larvae is no longer observed.

It is also apparent that these effects are not mediated by live yeast; no differential effect of A2 and A6 is shown on the growth of yeast (Figure 1) nor are the effects of A6 on *ee* changed by the use of SANG's defined medium, without live yeast (Table 4).

The fact that a shorter conditioning period (Table 3) produces a smaller reduction in survival rates adds to the argument that some agent accumulating in the waste products is responsible for the interaction. The sensitivity to the agent is found in larvae at least through 72 hours. The larvae may ingest the agent, or it may be absorbed during molting.

To determine whether this type of genotypic interaction occurs in other com-

petitive systems, *D. pseudoobscura*, a species that is usually eliminated when competing with *D. melanogaster* in population cages, was tested in homo- and heterotypic cultures with both A6 and A2 larvae. *D. pseudoobscura*-conditioned medium significantly improves the survivorship of A2 larvae over that of A2 on nonconditioned medium. Freezing of the medium adversely affects P1 fitness. However, P1 larvae are even further inhibited by A2-conditioned medium. In both cases, it is apparent that *D. pseudoobscura* larvae are much more sensitive than those of *D. melanogaster* to larval metabolic end-products in the surrounding medium.

Differences in metabolic end-products among organisms with apparent 'wild-type' phenotypes have been demonstrated in many species. The classic examples in man are the excretion of phenylpyruvic or homogentistic acid by certain genotypes. Through the use of such modern techniques as chromatography and electrophoresis, morphologically-unaffected biochemical mutants, have also been discovered in *Drosophila* (see WRIGHT 1963). Some of these genotypes probably modify the culture medium by excretion of inhibitory substances or by providing a deficient nutrient that improves the viability of commensal strains. It is certainly possible that two strains, each supplying a deficient precursor for the other, could establish a stable polymorphism. In polymorphic populations, BEARDMORE (1963) and BEARDMORE, DOBZHANSKY and PAVLOVSKY (1960) showed that with the increase in fitness produced by heterozygous superiority, there is a concomitant reduction in variance which could be explained on the basis of mutual facilitation of the various genotypes by a mechanism similar to that described in this paper.

Thus, the coexistence of certain strains or species in a given environment may not be fortuitous, but may result from complementation of nutritional requirements. Since the interaction between genotypes seems to be strain-specific, it should become apparent that experimentally determined relative fitnesses cannot be used to extrapolate the fitness of one population under one condition to other competitive conditions, a conclusion also indicated by STRICKBERGER (1963).

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SUMMARY

Interaction among competing strains or species of plants and animals, leading to the increase or decrease in survival rates of the commensal genotypes has been demonstrated by several investigators. The present work indicates that in at least two such cases—the interaction between larvae of *D. melanogaster* carrying ebony with either a naturally occurring or inbred wild-type strain, and also between larvae of *D. melanogaster* and *D. pseudoobscura*—the most reasonable explanation is that the waste products of each genotype modify the medium thereby affecting the growth of competing larvae. By growing larvae of one strain in the presence of accumulated metabolic products of other strains and measuring larval

survivorship by the number of flies eclosing, it is found that different biotic residues produce differential survival rates. Several such interactions have been demonstrated in the present work. In interspecific competition, the survival rate of *D. pseudoobscura* is decreased by the biotic wastes of a *D. melanogaster* wild type, and conversely, media conditioned by *D. pseudoobscura* significantly improved the viability of *D. melanogaster* as compared with pure cultures of *D. melanogaster*. Chemically mediated interactions among competing strains could possibly explain certain cases of balanced polymorphism (i.e., when each competitor complements the other's nutritional needs) and suggests that the occurrence of two sympatric species may not be fortuitous.

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