ENVIRONMENTAL DETERMINATION OF SELECTIVE SIGNIFICANCE OR NEUTRALITY OF AMYLASE VARIANTS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Strains homozygous at the amylase locus were derived from a polymorphic laboratory population of *Drosophila melanogaster*. The $Amy^{4,6}$ strain has higher enzyme activity than the Amy^{1} strain.——Maltose has the same nutritional value as starch.——The effect of starch in pure culture depends on the yeast level. At low yeast level increasing starch increases survival, at high yeast level increasing starch increases mean dry weight. The strains do not differ in survival or mean dry weight in pure culture.——In mixed cultures at 50% input of $Amy^{4,6}$ and Amy^{1} as larvae the percentage $Amy^{4,6}$ in adults increases with increasing starch at low yeast levels, but equals input frequency at high yeast levels. No increase in percentage $Amy^{4,6}$ in adults is present with increasing maltose at low yeast levels in mixed culture. The increase in percentage $Amy^{4,6}$ with increasing starch must be due to selection on the amylase locus working by competition for food in the larval stage. The single locus selection coefficient is determined by the environment and can reach quite high values.——Viability selection in the presence of starch is in the direction indicated by the enzyme activities.

SINCE the first observations, by HARRIS (1966) and LEWONTIN and HUBBY (1966) on the high proportion of enzyme loci that is polymorphic for electrophoretically distinguishable enzyme variants, the issue of selection or neutrality of enzyme variation has been under constant discussion (e.g. KIMURA and OHTA 1974, AYALA *et al.* 1974).

The evidence adduced is often open to two interpretations. Uniformity of gene frequencies over many subpopulations, for instance, can be attributed to balancing selection (PRAKASH, LEWONTIN and HUBBY 1969) or to neutrality of alleles and migration (MARUYAMA and KIMURA 1974). A correlation between gene frequencies and an environmental variable (KOEHN 1969) while indicative of selection is not necessarily caused by selection on the enzyme locus under consideration: the selection could have acted on linked loci. The same considerations apply to the work of LEWONTIN and KRAKAUER (1973). The fundamental problem is to isolate the effect of the difference at a single gene locus from the effect of variation at other loci. LEWONTIN (1974) supposes this problem to be unsolvable.

We think it can be solved in some cases by using changes in the environment

that are relevant and specific to the action of the enzyme under observation. The problem is essentially the problem of finding a suitable control. An enzyme system in which such is possible should satisfy the following conditions:

1) the substrate of the enzyme is known
2) the substrate level can be varied experimentally
3) the product of the enzyme is known
4) the product level can be varied experimentally.

These requirements are met by the amylase genotypes in *Drosophila melanogaster*. Amylase occurs in a number of tissues and in the hemolymph, but is present in high concentration in the posterior midgut of *D. melanogaster* larvae. Function of amylase in the posterior midgut is probably digestion of nutritional starch, but the function of the amylase in the hemolymph and the other tissues is unknown (DOANE 1969b). The amylase gene consists of two closely linked loci (distance .008 cM) at II-77.3 (BAHN 1968); these two loci have probably originated as a duplication. At both loci together a number of electrophoretically distinct isoenzymes were found (DOANE 1967, 1969a), numbered 1 to 6 from the anode to the origin. Because of the duplication either one or two isoenzymes are present in the homozygotes. The amylase activity in homogenates of homozygous flies differed in different isoenzyme genotypes.

The hypothesis to test is: a genotype with high amylase activity is better adapted to food containing starch than one with low amylase activity, and this will cause selection favouring the high activity genotype in competition for food. Increasing the concentration of starch in the food would then cause an increase in the fitness of the variant with high amylase activity relative to the variant with low activity. It is not sufficient to compare variants with different amylase activities at one level of starch. Such a comparison will always include effects of the genetic background, which will inevitably differ between the strains used in the comparison.

More specific information will be obtained by comparing the relative fitnesses at a range of starch concentrations. But even then an increase of relative fitness of the active amylase variant could be caused by aspecific effects, e.g. the total amount of food available. In order to circumvent the effect of the genetic background, it is necessary to compare the fitnesses of variants with different amylase activities on food in which the substrate is present and on food of the same nutritional value in which the product maltose is substituted for the substrate starch.

MATERIALS AND METHODS

The two variants used were $Amy^{4,6}$ and Amy^{1} homozygotes. Ten $Amy^{4,6}$ and 10 Amy^{1} strains were derived by single-pair crosses from a Kaduna wild-type population kept on cornmeal food (DE JONG *et al.* 1972). The ten strains of a type were left for several generations in a population cage to interbreed; experimental stocks were derived from these pure variant cages.

Amylase variants were electrophoretically assayed. Adult flies were homogenized in .025ml 0.1 M phosphate buffer, pH 7.5. Electrophoresis was for 3h on .9% agarose in 41 mM veronal buffer, pH 8.4, at 14 V/cm. Gels were incubated in a .2% boiled starch solution (Merck's soluble starch) in phosphate buffer, .025 M, pH 6.7, at 40° for 45 min. Staining was done in

I-KI solution (1M) for 15 minutes. Enzyme bands show up as transparent bands on a blue background.

The starch splitting activity of the alloenzymes was assayed by the dinitrosalicylic acid (DNSA) method of NOELTING and BERNFELD (1948), as described by DOANE (1967). This method measures the number of reducing groups formed. Assay of enzyme activity was done in a Tris-HCl buffer .01 M at pH 7.4, with .5% starch concentration and an enzyme dilution or maltose were considered to represent equal numbers of molecular glucose units.

Viability and competition experiments were done according to the method of BAKKER (1961). In 2.5 by 8 cm vials 5ml 2% agar was pipetted to prevent desiccation of the food medium. On top of this 5ml agar 2ml of 1% agar was pipetted containing the food. Food was given as:

1) killed yeast (dried bakers yeast, Engevita)
2) starch (Merck's soluble starch, nr. 1252, pro analysi)
3) maltose (Merck's nr. 5312, pro analysi).

Food media contained either yeast, (the pure yeast media), or yeast plus one of the two additional nutrients (starch and maltose media), in measured quantities. Equal weights of starch of ½ fly per ml. The flies were 4–5 days old.

In density series a range of yeast levels per larva was chosen, both unsupplemented and supplemented with starch or maltose in a weight proportion starch/maltose:yeast of 5:2.

In experiments on the properties of the amylase variants in pure culture the following levels of nutrients were used:

yeast	.1	.2	.4	.8	mg per larva
starch	.25	.5	1.0	2.0	mg per larva.

Combination of the four levels of these two nutrients gives 16 starch media for experiments with pure cultures. The density series runs diagonally through this 4×4 matrix of food media.

In experiments on the properties of the amylase variants in competition cultures the following levels of nutrients were used:

yeast	.1	.2	.4	.8	mg per larva
starch	.25	.5	1.0	2.0	mg per larva
maltose	.25	.5	1.0	2.0	mg per larva.

This yields 16 starch media (as in the pure culture experiments); the combination of the two lower yeast levels and the four maltose levels, and the combination of the highest yeast level and .25 and 2.0 mg maltose per larva gave 10 maltose media.

Flies were allowed to lay eggs for 4 hours on a gel of commercial starch (GALE 1964). Eggs were transferred to petri dishes with 2% agar gell for hatching. The peak of larval emergence is at 22 hours after egg-laying. Just after peak emergence, each experimental vial was stocked with 40 newly hatched larvae. At this number of larvae all the food presented is used. In each experiment all experimental vials were stocked within 2½ hours. Experiments were performed in a 25° climate room, 40–50% R.H.

EXPERIMENTS AND RESULTS

Enzyme activity

The enzymatic activity of amylase in adult *Drosophila melanogaster* differs with the variant. The $Amy^{4,6}$ experimental flies had an activity of $28.2 \times 10^{-3} \pm 1.6 \times 10^{-3}$ mg maltose/♂ fly/minute, the Amy^{1} experimental flies had an activity of $8.6 \times 10^{-3} \pm 1.6 \times 10^{-3}$ mg maltose/♂ fly/minute. Because of the higher enzyme activity of $Amy^{4,6}$ selection in favour of $Amy^{4,6}$ can be expected in the presence of starch.

Density series

The first question is whether it is really starch that is used as food and not a

TABLE 1

Sterile density series : Percent viability

	mg yeast per larva							
	.16	.20	.32	.40	.50	.62	.80	1.60
Without starch	1	0	1	10	12	30	41	38
With starch	6	7	12	56	56	60	60	50

product of bacterial degradation. This was investigated by rearing larvae under sterile conditions in a series of experimental vials with increasing amounts of yeast per larva, both without starch and supplemented with starch in a weight proportion starch:yeast of 5:2. The output of the starch vials as numbers of adults was indeed higher than for the pure yeast vials over a considerable range of low yeast levels (Table 1).

Secondly, it is shown that starch and maltose have a comparable nutritional value. In density series both numbers of adults and mean dry weight of adults are more similar when reared on food media supplemented with maltose or starch than when either maltose or starch media are compared with pure yeast media (Figure 1). For further work the levels of .1, .2, .4, .8 mg yeast per larva

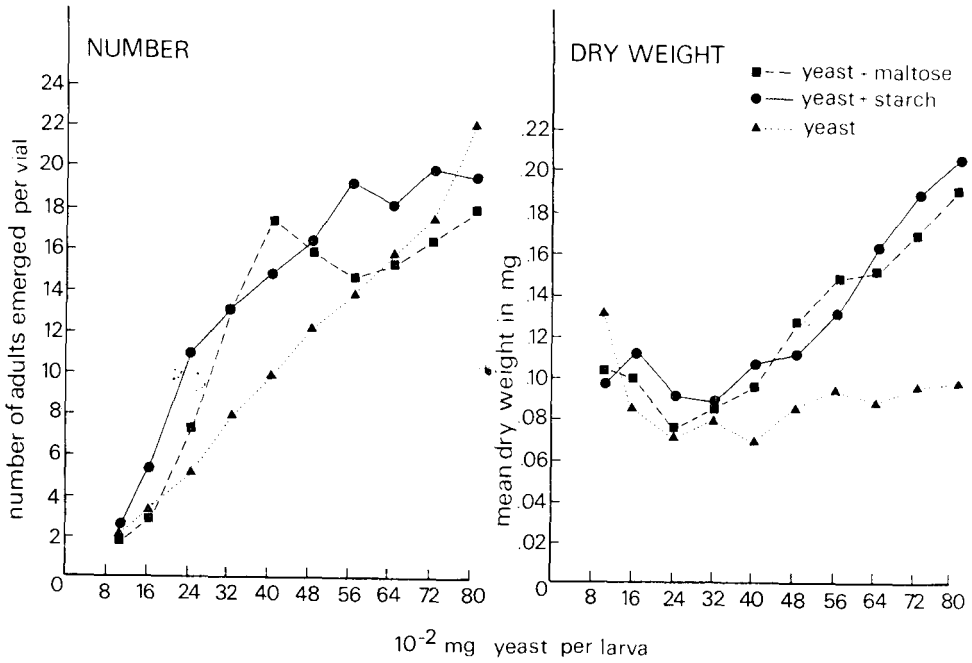

FIGURE 1.—Comparison of the effect of addition of starch and maltose on number and mean dry weight of adults. Of both the *Amy*^{1} and the *Amy*^{4,6} strain 16 pure cultures were scored at each food concentration. As no differences existed between the strains the results of the *Amy*^{1} and *Amy*^{4,6} strains were combined before converting to number of adults and mean weight per vial.

were chosen on basis of the density series. At the two lower yeast levels larval mortality is considerable, at the higher yeast level further addition of yeast does not improve larval survival.

Pure cultures on starch media

On each of the 16 starch media 10 replicate vials were run for each variant, $Amy^{4,6}$ and Amy^{1}. The numbers and mean dry weights of emerging adults were compared.

The numbers of adults emerged from a total of 400 larvae per starch medium

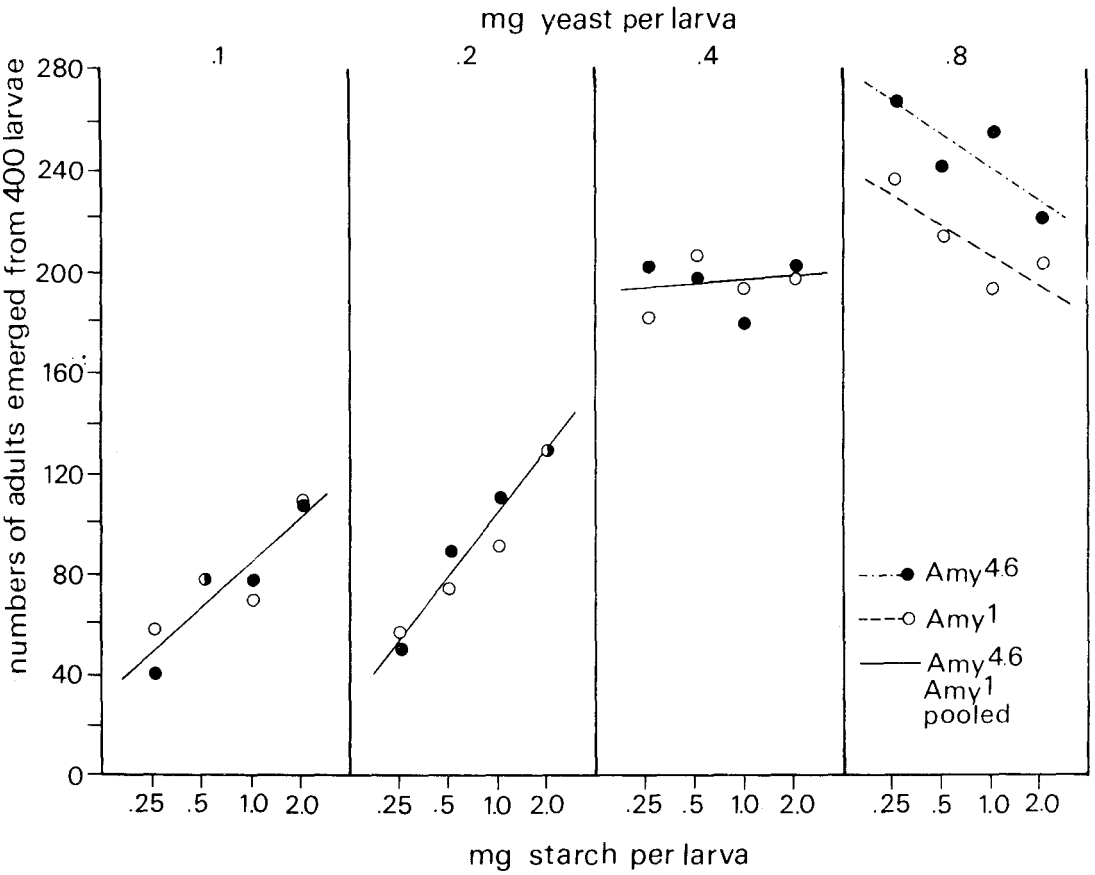

FIGURE 2.—Number of adults emerged from pure culture of the Amy^{1} (○) and from pure culture of the $Amy^{4,6}$ (●) strain on food containing starch. Difference between the strains in emergence exists only at the .8 mg yeast per larva level. The total number of adults increases with yeast level. The regression coefficients of number of adults on starch level are, per yeast level:

.1 mg yeast per larva:	$b = 16.9 \pm 3.5$	$.001 < P < .01$
.2 mg yeast per larva:	$b = 24.6 \pm 2.0$	$P < .001$
.4 mg yeast per larva:	$b = 1.4 \pm 2.7$	ns
.8 mg yeast per larva:	$b_1 = -12.5 \pm 5.3$	ns
	$b_{4,6} = -12.3 \pm 6.2$	ns.

TABLE 2

Analysis of variance. Number of adults emerged in pure culture

	SS	df	MS	F	P
Yeast	137990.094	3	45996.698	472.282	$P<.001$
Starch	2598.594	3	866.198	8.894	$.001<P<.005$
Genotype	850.781	1	850.781	8.736	$.01<P<.025$
Yeast × Starch	8724.281	9	969.365	9.953	$.001<P<.005$
Yeast × Genotype	1722.844	3	574.281	5.897	$.01<P<.02$
Starch × Genotype	309.344	3	103.115	1.059	not significant
Within	876.531	9	97.392		
Total	153072.469	31			

Note the high interaction Yeast × Starch.
The Yeast × Genotype interaction is solely due to .8 mg yeast per larva differing from the other yeast concentrations, as is the genotype difference itself (see Table 3).

increased strongly with yeast level (Figure 2, Table 2). The starch × yeast interaction in an analysis of variance on number was highly significant. An ensuing analysis of covariance (Table 3) revealed that at the two lower yeast levels, .1 and .2 mg yeast per larva, the number of adults emerged increased significantly with the amount of starch present. At the two higher yeast levels (.4 and .8 mg yeast per larva) no increase in the number of adults was present (Figure 2). At .1, .2 and .4 mg yeast per larva no difference in emerging number of adults exists between $Amy^{4,6}$ and Amy^{1} in pure cultures. The significant difference between genotypes and the significant yeast × genotypes interaction in the analysis of variance are solely due to the higher emergence of $Amy^{4,6}$ at .8 mg yeast per larva. This difference is, however, the same for all starch concentrations. Therefore it cannot be attributed to digestive action of $Amy^{4,6}$ in a starch environment; it is an effect of the genetic background or an effect of the amylase locus in a nondigestive function.

As males and females have different weights, mean dry weight of emerging adults was determined separately for the two sexes. Between amylase variants no significant differences in mean dry weight were found. At the two lower yeast levels, .1 and .2 mg, mean dry weight was independent of starch level. At the two higher yeast levels, .4 and .8 mg per larva, mean dry weight increased significantly with starch level (Figure 3). At .4 mg yeast per larva the overall mean dry weight was not significantly higher than at .1 and .2 mg yeast per larva, but at .8 mg yeast per larva the overall mean dry weight is significantly higher than at the three other yeast levels. In this respect no difference exists between sexes.

It must be concluded that in pure culture no differences were found between the strains with different amylase variants that could be attributed to the amylase locus. But it is shown that the amount of starch is an environmental factor pertinent to Drosophila. The nutritional background determines what effect starch will have. Depending on the amount of yeast that is used as background nutritional level, the influence of an increasing amount of starch is in survival or in

TABLE 3

Analysis of number of adults in pure culture

Yeast level in mg/larva	Genotype	Genotypes per yeast level: Differences in slope $F_{1,4}$	P	Differences in height $F_{1,5}$	P	Total slope $b \pm s_b$	P	Yeast levels: Differences between slopes $F_{1,12}$	P	Difference in total number of adults in analysis in variance $F_{1,9}$	P
.1	*Amy*^{4,6}	.602	ns	.149	ns	16.9 ± 3.5	.001<P<.01				
	Amy^{1}							3.299	.10 ns	8.487	.01<P<.025
.2	*Amy*^{4,6}	.293	ns	1.704	ns	24.6 ± 2.0	P<.001				
	Amy^{1}										
.4	*Amy*^{4,6}	.348	ns	.222	ns	1.4 ± 2.7	ns	42.785	P<.001	462.808	P<.001
	Amy^{1}										
.8	*Amy*^{4,6}	.0006	ns	18.452	.005<P<.01	−12.5 ± 5.3	ns	3.231	.10 ns	45.407	P<.001
	Amy^{1}					−12.3 ± 6.2	ns				

Equality of the genotypes in regression of number of adults on starch level, by analysis of covariance.
ns = not significant.

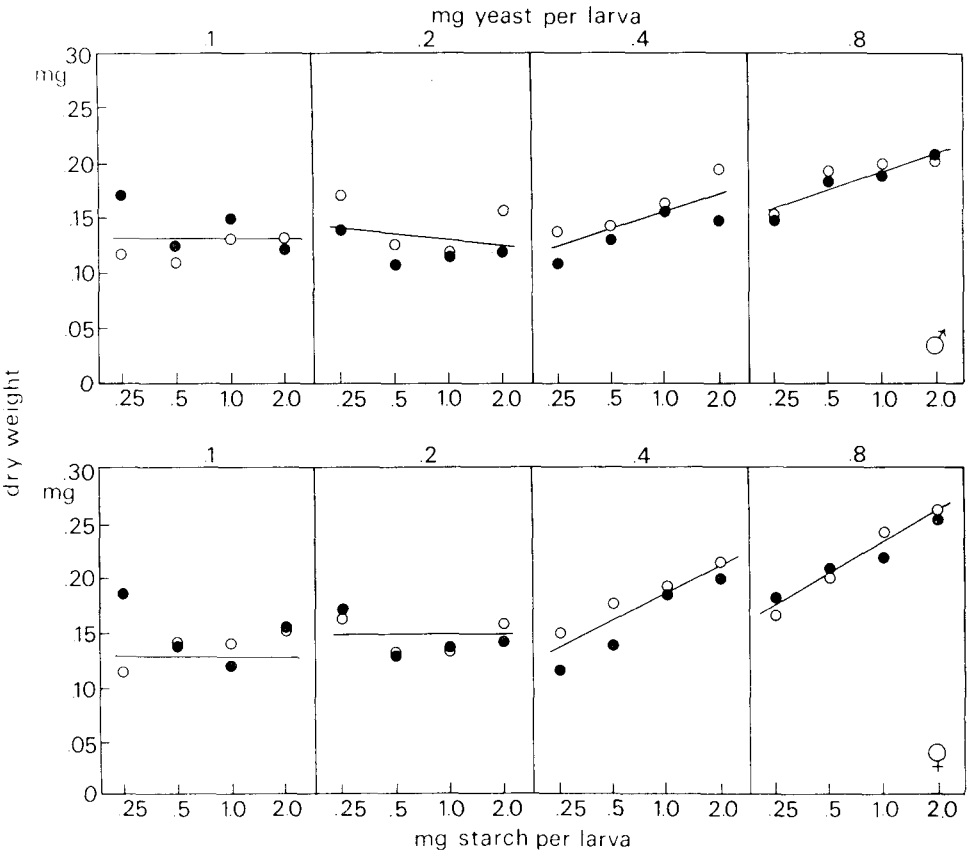

FIGURE 3.—Mean dry weight of adults emerged from pure culture of the *Amy*^{1} (○) and from pure culture of the *Amy*^{4,6} (●) strain on food containing starch. No difference between the strains exists in mean dry weight in either males or females. In both males and females mean dry weight is significantly higher on .8 mg yeast per larva than on the other three yeast levels. The regression coefficients of mean dry weight on starch level are, with increasing yeast levels, for males:

-2.5 ± 9.5 (ns), -5.2 ± 9.0 (ns), 16.4 ± 5.5 ($P \cong .01$), 17.6 ± 4.1 ($P < .001$), and for females:

$.3 \pm 10.7$ (ns), -4.9 ± 8.1 (ns), 26.0 ± 6.3 ($.001 < P < .01$), 29.5 ± 3.5 ($P < .001$).

dry weight: at the lower yeast levels the number of adults increases with the amount of starch presented to the larvae, at the higher yeast levels mean dry weight increases with the amount of starch. As a consequence, biomass of adults increases with the amount of starch presented to the larvae for all four yeast levels (Figure 4).

Competition for food between Amy^{4,6} *and* Amy^{1} *larvae*

To test for differential survival of the amylase variants in larval competition, each culture was stocked with 20 *Amy*^{4,6} and 20 *Amy*^{1} larvae. In addition to the same 16 starch media as in the pure culture experiment, 10 maltose media were

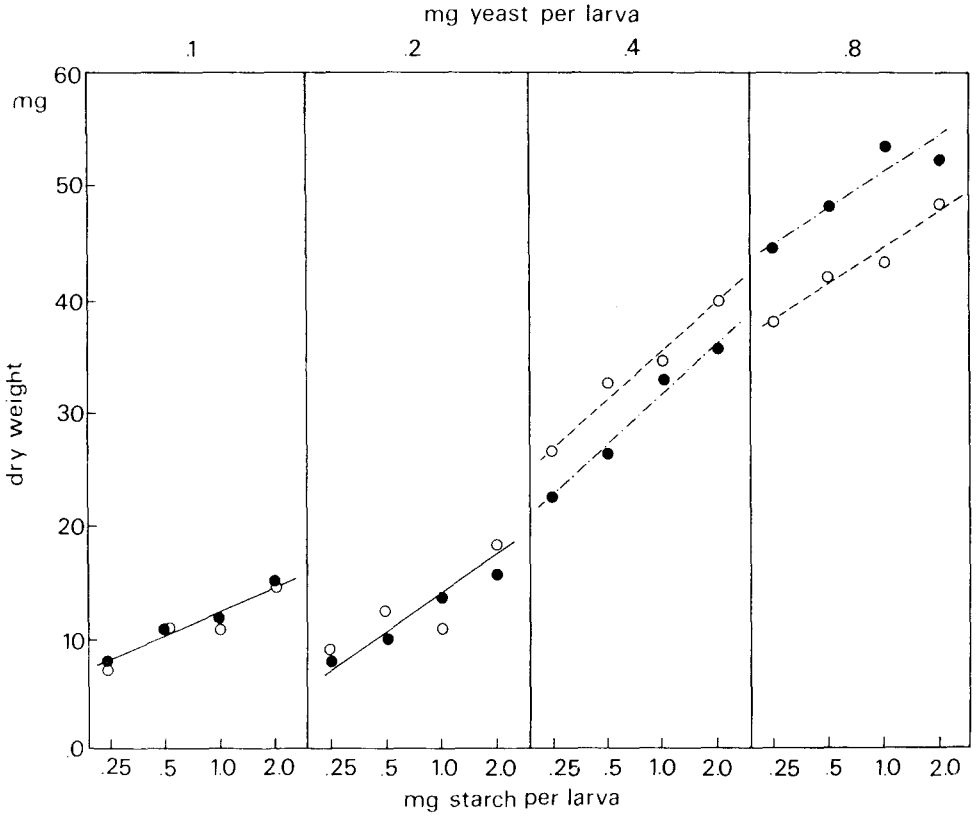

FIGURE 4.—Total dry weight of adults emerged from pure culture of the Amy^1 (○) and from pure culture of the $Amy^{4,6}$ (●) strain, on food containing starch. Only at .8 mg yeast per larva no differences in total dry weight exist between the strains, due to the higher emergence of $Amy^{4,6}$ at that yeast level. The regression coefficients of total dry weight on starch level are:

.1 mg yeast per larva	$b = 22.35 \pm 2.74$	$P < .001$
.2 mg yeast per larva	$b = 25.65 \pm 5.22$	$.001 < P < .01$
.4 mg yeast per larva	$b = 43.80 \pm 5.46$	$P < .001$
.8 mg yeast per larva	$b_1 = 32.50 \pm 4.26$	$.001 < P < .01$
	$b_{4,6} = 29.50 \pm 9.31$	$P \cong .05$

used, 4 at each of the two lower levels, and two (.8/.25 and .8/2.0) at the highest yeast level. At the two lower levels 30 vials were run for each medium, at the two higher yeast levels 20 vials were used for the starch and 16 for the two maltose media.

In the maltose media the nutritional level is enhanced by adding the product of the enzymatic degradation of starch. Any influence on amylase frequencies in the adults by the addition of maltose to the larval food must be due to factors other than the digestion of starch: it cannot be due to selection acting on the amylase locus. In the maltose cultures the amylase variants are expected to be selectively neutral with respect to starch digestion by the irrelevance of the environment to the amylase locus under observation.

Starch is a relevant environmental factor for *Drosophila melanogaster* larvae in general, as shown in the pure culture experiments. Now, as $Amy^{4,6}$ has a higher enzyme activity than Amy^{1}, the possibility of selection is present. Let us see whether selection occurs.

The numbers of adults emerging from competition cultures at .1 and .2 mg yeast per larva did not differ between starch and maltose media (Table 4). This confirms the result obtained in the density series that the nutritional values of starch and maltose are equal.

The proportion of $Amy^{4,6}$ *in adults depends on the food medium*

In Table 4 the results for all starch and maltose media are given. The same results, as percentage $Amy^{4,6}$ emerged (converted to degrees of arc) are shown in Figure 5. The same picture emerges from G-tests (SOKAL and ROHLF 1969) on the original data and from an analysis of covariance on the transformed percentages.

At .1 mg yeast per larva the slope of the regression line of the transformed percentages $Amy^{4,6}$ on starch level is positive and significantly different from zero (Figure 5). For the maltose cultures at .1 mg yeast per larva the picture is different: although the slope is nearly significantly different from zero, it is much smaller. Between starch and maltose media at .1 mg yeast per larva significant heterogeneity exists ($.01 < P < .02$) as well as a significant difference in the slope of the regression of transformed percentage $Amy^{4,6}$ on the level of the added nutrient ($.01 < P < .05$).

At .2 mg yeast per larva, the regression of transformed percentage $Amy^{4,6}$ on starch level is not significant. Yet the positioning of the points (Figure 5) is suggestive of a positive slope. In accordance with a positive slope, heterogeneity between the starch media at .1 and .2 mg yeast per larva is low ($.30 < P < .50$); nor are there any differences in slope ($.50 < P < .75$) or height ($.10 < P < .25$) in an analysis of covariance. For the maltose cultures at .2 mg yeast per larva regression of percentage $Amy^{4,6}$ in adults on maltose level is not significantly different from 0 (Figure 5). At .2 mg yeast per larva no significant difference between starch and maltose exists. The fourth comparison, maltose media at .1 mg yeast per larva with maltose media at .2 mg yeast per larva, shows a significant heterogeneity ($.001 < P < .01$) and a significant difference in height ($.001 < P < .01$), but not in slope ($.50 < P < .75$), of the regression lines of percentage $Amy^{4,6}$ on nutrient level.

At .4 and .8 mg yeast per larva, the gene frequencies in emerging adults are not affected by the starch level (Table 4 and Figure 5) and equal the input frequencies. This is also the case in the two maltose media at .2 mg yeast per larva. While no heterogeneity exists between the numbers $Amy^{4,6}$ and Amy^{1} emerged in starch cultures of .1 and .2 mg yeast per larva ($.30 < P < .50$) nor between the numbers $Amy^{4,6}$ and Amy^{1} emerged in starch cultures of .4 and .8 mg yeast per larva ($P \simeq .50$), a highly significant heterogeneity exists between .1 and .2 mg yeast per larva on the one hand, and .4 and .8 mg yeast per larva on the other hand ($P < .001$). This is indeed the same division in reaction on nutrient level

TABLE 4

Numbers of Amy^{4,6} *and* Amy^{1} *adults emerged from larval competition, together with G tests at 1 df for agreement to input 1:1 ratios. The relative viability (v) of* Amy^{4,6} *to* Amy^{1} *is also given.*

Yeast level mg/larva	Nutrient mg/larva	Starch						Maltose					
		Number of vials	*Amy*^{4,6}	*Amy*^{1}	G	P	v	Number of vials	*Amy*^{4,6}	*Amy*^{1}	G	P	v
.1	.25	30	68	127	18.135	***	.54	30	60	114	17.040	***	.53
	.5	30	90	126	6.031	*	.71	30	72	137	20.556	***	.53
	1.0	30	89	116	3.568	ns	.77	30	86	143	14.339	***	.60
	2.0	30	118	132	.787	ns	.89	30	88	143	13.223	***	.62
.2	.25	30	99	151	10.897	***	.66	30	123	166	6.424	**	.74
	.5	30	85	127	8.378	**	.67	30	116	171	10.605	***	.68
	1.0	30	111	113	.019	ns	.98	30	131	156	2.183	ns	.84
	2.0	30	152	170	1.009	ns	.90	30	144	181	4.223	*	.80
.4	.25	20	211	185	1.711	ns	1.14						
	.5	20	192	227	2.929	ns	.85						
	1.0	20	192	214	1.196	ns	.90						
	2.0	20	218	194	1.400	ns	1.12						
.8	.25	20	249	265	.501	ns	.94	16	127	139	.543	ns	.91
	.5	20	226	233	.109	ns	.97						
	1.0	20	230	238	.139	ns	.97						
	2.0	20	213	233	.900	ns	.91	16	120	129	.322	ns	.93

*** $P<.001$
** $.001<P<.01$
* $.01<P<.05$

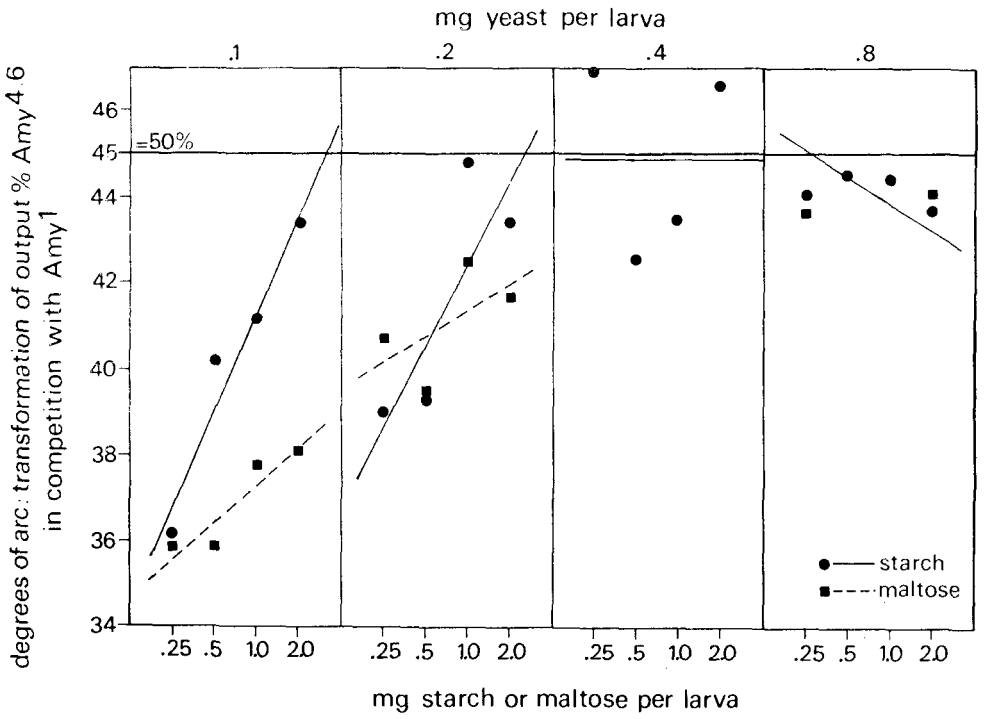

FIGURE 5.—Percentage $Amy^{4,6}$ emerging from competition with Amy^{1} at initial frequencies of 50% $Amy^{4,6}$ and Amy^{1}. Percentages are transformed into angles by the arc sine transformation.

The regression coëfficients of transformed percentages $Amy^{4,6}$ on the concentration of the additional nutrient, starch or maltose, are:

for starch:

.1 mg yeast per larva	b =	2.25 ± .42	(.01 < P < .05)*
.2 mg yeast per larva	b =	1.87 ± .89	(.10 < P < .20)
.4 mg yeast per larva	b =	.02 ± 1.20	(P > .9)
.8 mg yeast per larva	b =	— .12 ± .25	(.60 < P < .70)

for maltose:

.1 mg yeast per larva	b =	.84 ± .26	(.05 < P < .10)
.2 mg yeast per larva	b =	.60 ± .58	(.40 < P < .50)

A significant difference in slope exists between starch and maltose cultures at .1 mg yeast per larva (.01 < P < .05).

of yeast as was present in the number count and the mean dry weight count in the pure culture experiment.

At those yeast levels (.1 and .2 mg per larva) at which starch has an influence on survival of *Drosophila melanogaster* in pure culture, selection is favouring the high enzyme activity gene $Amy^{4,6}$ in a mixed culture of $Amy^{4,6}$ and Amy^{1}. This is attested to by the *difference* in slope of the $Amy^{4,6}$ percentage in emerging adults on the starch level and on the maltose level in the larval environment. Clearly a relation exists between the relevance of the environmental variable starch level to survival and the occurrence of viability selection. It is to be noted

that the different relative viability of the two variants in competition is not reflected in pure cultures: the emergence in pure culture is the same for both types. At the two higher yeast levels, .4 and .8 mg yeast per larva, starch has no influence on survival of *Drosophila melanogaster*, but on mean dry weight. In accordance with this lack of relevance to survival in pure culture, at these levels no viability selection is found. At these yeast levels the amylase genotypes $Amy^{4,6}$ and Amy^{1} are selectively neutral in viability despite the difference in enzyme activity.

DISCUSSION

The work on electrophoretic variability has shown enormous reservoirs of genetic variability in populations. But the significance of this type of variability for evolutionary change as we know it from the palaeontological record can only be evaluated after it has become clear what part of it is directly subjected to selection. At present we have almost no evidence that electrophoretic variation has biological significance, i.e. that electrophoretic variation affects fitness by way of morphology, physiology or behaviour.

It is conceivable that most of the variation relevant to evolutionary changes does not reside in the structural part of the genome, but involves the regulatory part. Therefore it is of the utmost importance to establish whether electrophoretic variation is selected.

In most studies that show effects of selection on enzyme loci (e.g. SING, BREWER and THIRTLE 1973) it is impossible to decide whether selection is on the genetic background or on the locus itself. Selection on the locus itself seems the more likely when some enzyme property as temperature stability can be correlated with environmental temperatures (KOEHN 1969), although this does not logically exclude the possibility of selection working on a linked temperature-sensitive locus. The same situation is found for alcohol dehydrogenase in *Drosophila melanogaster*. The balance of the evidence (VIGUE and JOHNSON 1973; DAY, HILLIER and CLARKE 1974ab; BIJLSMA-MEELES and VAN DELDEN 1974) is in favour of selection on the locus itself. The locus is probably kept polymorphic by varying direction of selection in different environments, the fast allozyme being more active, and the slow allozyme being more stable.

Single gene heterosis has been claimed for octanol dehydrogenase in *D. pseudoobscura* (WILLS and NICHOLS 1971, 1972). This has been severely criticized (YAMAZAKI 1972; KIDWELL 1974), since it only seemed to appear in highly inbred lines and in part of the experiment. Moreover, as no enzyme activities of octanol dehydrogenase allozymes were given, it is impossible to correlated genotype frequency change with enzyme characteristics.

In at least one case, esterase −5 in *D. pseudoobsura*, no selection could be detected in extensive fitness experiments (YAMAZAKI 1971). In some cases gene frequencies over many generations did not show any influence of selection (examples in MACINTYRE and WRIGHT 1966; SING, BREWER and THIRTLE 1973; POWELL 1973).

The scarcity of observed and well documented selection on single enzyme loci

induced us to investigate selection on the amylase locus in *Drosophila melanogaster*. After we found that in a number of laboratory populations the frequencies of amylase variants were related to the presence of starch in the food, we decided upon an ecological rather than a standard population genetics approach.

Firstly, it is shown that starch is a relevant environmental factor for *Drosophila melanogaster*. At low yeast levels addition of starch promotes survival of the larvae. While at the low yeast levels starch has no influence on the weight attained by the adults, at high yeast levels addition of starch furthers an increase in the weight reached by the adults. At these high yeast levels starch has no longer any influence on survival.

Secondly, the great difference in enzyme activity between homozygous amylase genotypes leads us to suppose that starch is digested at different rates by larvae differing in their amylase genotype. Then the genotype with the higher enzyme activity, $Amy^{4,6}$, will gain an advantage over Amy^{1}. This advantage will result in an increase in fitness for the $Amy^{4,6}$ genotype. This fitness effect will increase with increasing amounts of starch in the food medium. The fitness component which will change will be determined by the general nutritional level. At low yeast levels one will expect viability selection, because of the relevance of starch to viability in pure culture. At high yeast levels there is scope for fertility selection because of the relevance of starch to weight in pure culture. Fertility is positively correlated with weight in *Drosophila melanogaster* (ROBERTSON 1957).

No difference exists between Amy^{1} and $Amy^{4,6}$ strains in survival or weight in pure cultures. It had for other characteristics already clearly been shown that pure culture viability and competitive performance are not related in *Drosophila melanogaster* (BAKKER 1961, 1969; LEWONTIN 1955). BAKKER gave an ecological explanation of this phenomenon, a theoretical explanation is given in a model by DE JONG (1976, and in preparation). However, a prediction as to the fitness component affected by the process of competition can be based on the comparison of a range of food media, and a prediction as to the direction of selection can be based on the function difference of the enzymes.

A third very important point is where to start measuring selection from. The zero-point of no selection for the amylase *strains* is obviously that frequencies after competition equal input frequencies of the strains. But this is not the zero-point for evaluating selection on the amylase-*locus*. Selection on the amylase locus itself via digestive function can only be present if opportunity for differential digestive functioning of the enzymes $Amy^{4,6}$ and Amy^{1} exists, that is in the presence of starch. If maltose is present in the food, there is nothing for a digestive amylase to work on. Of course, amylase might have other metabolic functions than digestion in the gut, but when equivalent amounts of starch and maltose were given, any possible further differences cannot be distinguished from all other strain differences. Since the differences between the presence of the enzyme substrate and the enzyme product is the enzyme action, and selection on amylases should mean a consequence of the enzyme action, the zero-point to start measuring selection on the amylase *locus* from is viability of the strains in

the maltose competition cultures. Selection on the amylase locus in the presence of starch is the difference between maltose and starch competition cultures. The actual values of the viabilities of the strains in maltose competition cultures are determined by all other differences between the strains, including besides the genetic background other functions of amylase. What these actual values are is irrelevant to the method.

The outcome of the competition experiments agrees with the notions of environmental relevance and functional variation; the latter if our *a priori* point is taken regarding where to measure selection on the *locus* from.

We have shown that at high yeast levels no difference in viability in competition exists between the strains, while at low yeast levels, where total viability in pure and mixed culture is influenced by adding starch to the yeast, a difference exists between the strains in competition. The Amy^1 strain is favoured in competition at low yeast levels. The increase in viability of the $Amy^{4,6}$ strain with increasing levels of starch is in itself no proof of selection on the *Amy* locus. It could be a consequence of improved general food condition, which makes total viability higher, and therefore the scope for selection smaller. But if this is true, we would expect that the same improvement in general food conditions as obtained by adding equivalent amounts of maltose leads to the same result. That is, we would expect the same regression of relative viability of the $Amy^{4,6}$ strain on maltose as on starch. In fact, we found a small, not significant, regression that might indicate some effect of total food level, but the main result is the significant difference between the regressions of the relative viability of the $Amy^{4,6}$ strain on maltose and on starch. It is this difference in slope that can only be attributed to a difference in efficiency of digestion of starch between our Amy^1 and $Amy^{4,6}$ strains. The difference in slope shows the importance of varying the intensity of the putative selective agent, here starch, in order to determine whether selection can be present.

The change in proportion $Amy^{4,6}$ present in adults is very large at the level of .1 mg yeast per larva. The relative viabilities of the $Amy^{4,6}$ strain change from .54 to .90 with increased starch level. In comparing the highest maltose with the highest starch level we find an increase in relative viability of the $Amy^{4,6}$ strain from .62 on maltose to .90 on starch. This *difference* of .28 in relative viability is due to selection by the environmental factor starch at the amylase *locus*. Single-locus selection coefficients need not at all be small.

In the debate whether genetic variation is maintained by balanced selection or is the result of random drift, the proponents of drift assume the variation to be selectively neutral (KIMURA and OHTA 1974) or even slightly deleterious (OHTA 1974). Selective neutrality can originate in different ways. Functional neutrality is the absence of differences in the characteristics of the enzymes the alleles code for. The proponents of selective neutrality mostly seem to have this type of neutrality in mind. Amylase enzyme variants are not functionally neutral, they differ in activity, yet this does not necessarily mean they are being selected. We found three situations with respect to viability selection on the amylase locus.

1) At high yeast levels, i.e. yeast levels greater than the minimum of food necessary for survival, no viability selection is present: input and output frequencies are the same. This is the case both in the presence of starch and of maltose. Amylase variants are selectively neutral with regard to viability selection at these high yeast levels, even in the presence of the substrate starch and although they functionally differ. This is neutrality with respect to a fitness component that is unvarying in the prevailing environment. In this type of situation, genetic background, with the exception of mutants subject to "hard" selection, is not important. Input frequencies will be output frequencies whatever the genetic background, as the maximum number of larvae survives anyhow. Let us call this *general environmental neutrality*.

2) If not starch but maltose is present, amylase variants are selectively neutral because of the absence of the substrate in the environment. But dependent on the general nutritional level, viability can be the fitness component under environmental control. If it is, genetic background effects will determine what happens with the amylase genotypes, whether they are taken along with selected loci or not. Let us call this *specific environmental neutrality*.

3) If starch is present and viability is under environmental control, viability selection on the amylase locus itself is present and can be quite strong.

The occurrence of these three selective situations has a number of implications both for experiments and field work designed to detect selection. In experiments, care should be taken to identify the environmental component the enzymes functionally relate to, and to find the conditions in which the environmental component works on fitness before starting to measure selection coefficients. In the case of amylase, fitness experiments on normal culture food like cornmeal—molasses might very well not detect any selection. As to nature, let us consider an example in which we will use the selection coefficients of this experiment. Suppose two large *Drosophila melanogaster* populations of similar gene frequencies and similar genetic background but without migration. Both live on an ample supply of sugars and yeast, i.e. we suppose specific environmental neutrality. One generation both populations crash by lack of food. In the one population a few larvae survive on sugar, and in the other a few larvae survive on starch food. Viability selection is intense. Thereafter both populations enjoy their former plenty. The populations differ now in their amylase frequencies but an investigator would not be able to find any difference in ecology. Indeed, there would be none. Chance events in the environment of very short duration may have a definitive influence on the frequencies found at some later time. This is true even if the populations are almost always large and the selection coefficients almost always zero. HALDANE (1949) suggested the same situation for genes involved in resistance against infectious diseases.

Chance fluctuations of selection coefficients might be more important in nature than small selective coefficients. KARLIN and LIEBERMAN (1974), in their comprehensive treatment of random selection coefficients, point out that varying selection coefficients tend to preserve polymorphism. This is true even when selection coefficients have a mean of zero, making the phenotypes on the average

selectively neutral. Polymorphism is preserved if the variances of the selection coefficients are large enough. This type of model might very well be applicable to the amylase polymorphism and to the alcohol dehydrogenase polymorphism in *Drosophila melanogaster*.

Two separate questions remain. The first is what proportion of the observed variation can be selected. If we want to know this we have to disprove the three types of neutrality mentioned for a random sample of the observed varying loci. At each locus we have to look whether the electrophoretic variants are functionally different, whether they are related to an environmental constraint and whether they have a relation to fitness. The second question is what distribution selection coefficients have in nature in time and in space, and the relevance to evolution of changes in this distribution of selection coefficients. Both questions can only be answered in a thorough study of the ecology of the organism and its functional relation to the environment.

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