POPULATION GENETICS OF DROSOPHILA AMYLASE. IV. SELECTION IN LABORATORY POPULATIONS MAINTAINED ON DIFFERENT CARBOHYDRATES

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ABSTRACT

Two polymorphic systems impinging on α-amylase in *Drosophila pseudoobscura* have been studied in laboratory populations maintained on medium in which the only carbohydrate source was starch (the substrate of amylase) and replicas maintained on medium in which the only carbohydrate source was maltose (the product of amylase). The two polymorphic systems were alleles at the structural gene (*Amy*) coding for the enzyme (allozymes) and variation in the tissue-specific expression along the adult midgut controlled by several genes. In the seven populations on maltose medium little consistent change was noted in either system. In the seven populations on starch medium, both polymorphisms exhibited selective changes. A midgut pattern of very limited expression of amylase rose in frequency in all starch populations, as did the frequency of the "fast" (1.00) *Amy* allele. The overall specific amylase activity did not differ between starch-adapted and maltose-adapted flies.——The results, along with previous studies, indicate that when a gene-enzyme system is specifically stressed in laboratory populations, allozymes often exhibit selective differences. Such results make the selectionist hypothesis at least tenable. Furthermore, the fact that both types of polymorphisms responded to selection indicates the role of structural gene vs. gene regulation changes in adaptive evolution is not an either/or question but one of relative roles and interactions.

IT is agreed that measuring the effects of natural selection at a single gene locus is very difficult. One approach which may circumvent some of the difficulties is to use techniques analogous to those used by bacterial geneticists to select for "nutritional" mutants. The medium on which an organism is growing is altered in such a way that a physiological function of an enzyme encoded by a gene is specifically selected for or against. The adaptive response of a population to a particular physiological stress may be of at least two sorts. First, the gene(s) encoding the enzyme(s) needed to handle the stress may evolve to produce a more suitable enzyme via amino acid substitutions. Second, the enzyme may remain unchanged but its regulation changed so it is produced in appropriate amounts in the right tissue at the right time. Both types of responses have been observed, for example, in yeast stressed by phosphate depletion (FRANCIS and HANSCHE 1973; HANSCHE 1975).

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For several years our laboratory has been studying the population genetics of α-amylase (EC 3.2.1.1, α-1,4-gluconohydrolase) in Drosophila, primarily *D. pseudoobscura* (POWELL and LICHTENFELS 1979; POWELL 1979; POWELL, RICO and ANDJELKOVIĆ 1980). We have been especially interested in comparing the evolutionary dynamics of structural gene variants (allozymes) and variation in tissue-specific expression of the gene, *i.e.*, gene regulation variation. ABRAHAM and DOANE (1978) were the first to discover that variation in tissue-specific expression of Drosophila amylase along the adult midgut is under genetic control. We have confirmed that natural populations of *D. pseudoobscura* are polymorphic for amylase expression (POWELL 1979) and that the variation is to a large degree under genetic control (POWELL and LICHTENFELS 1979). We report here the response of laboratory populations of *D. pseudoobscura* maintained on medium in which the only carbohydrate source is starch (the substrate of α-amylase) and replicas on medium in which the only carbohydrate source is maltose (product). The frequencies of both the structural gene variants and tissue-specific expression patterns have been monitored.

MATERIALS AND METHODS

The flies used in the experiments reported here were all from Bryce Canyon, UT. The structural gene of α-amylase, *Amy*, is on the third chromosome of *D. pseudoobscura* and is nonrandomly associated with inversions in this chromosome (PRAKASH and LEWONTIN 1968; POWELL 1979). Therefore, it was important to be certain our populations were not segregating for chromosome variants. Ten or more larvae from each strain used were karyotyped by examination of salivary gland polytene chromosomes. Only strains monomorphic for the Arrowhead (AR) gene arrangement were used, Bryce Canyon being more than 95% AR. Periodically, the population cages were again checked for chromosomes to determine whether the initial screen had eliminated all variants or whether contamination occurred. In the first experiment two of eight populations were found to be polymorphic with Standard (ST); the total of 14 other populations have always been monomorphic for AR. Results from the chromosomally polymorphic cages are omitted.

The basic design of the experiments is illustrated in Figure 1. In experiment I, eight isofemale lines were mixed in different proportions to start the population cages. In experiment II mass cultures begun by several hundred females, caught at Bryce about 2 years after the first set, were used. These were mixed, and all cages started from the same base population. Each cage was begun by a minimum of 500 adults. The type of population cage used supports a population of more than 3000 adults. Three media were used in the course of the experiments. The starch medium was 3% soluble starch, 5% inactivated yeast, 1.5% agar and 0.6% proprionic acid. The maltose medium substituted 5% maltose for the starch. The amount of mold inhibitor (proprionic acid) is higher than usually used because these media were particularly susceptible to microorganism infection. Egg samples were reared on a "standard" cornmeal-molasses-yeast medium.

Allozyme frequencies were determined by acrylamide gel electrophoresis followed by soaking in a starch solution and then in an I_2-KI solution; areas of amylase activity are white bands on a dark blue background. The method to detect the pattern of amylase activity along the gut has been detailed by ABRAHAM and DOANE (1978). Briefly, this involves dissecting out the midgut of a 2- to 6-day-old adult and placing it on a microscope slide; a thin film of starch-agar in buffer is layered over the gut. After 10 minutes at room temperature, it is fixed and stained with iodine. Clear areas on these whole mount preparations indicate presence of α-amylase activity, whereas dark blue indicates lack of, or at least much reduced, activity. In *D. pseudoobscura* a total of 13 different patterns of activity have been observed (POWELL 1979), eight of which were reasonably common in the Bryce Canyon population studied here. The method of designating the patterns of activity has been described (DOANE 1980; POWELL and LICHTENFELS 1979). The anterior midgut (AMG) is divided into three regions designated 1, 2, and 3; the posterior midgut (PMG) is divided into two regions, 1

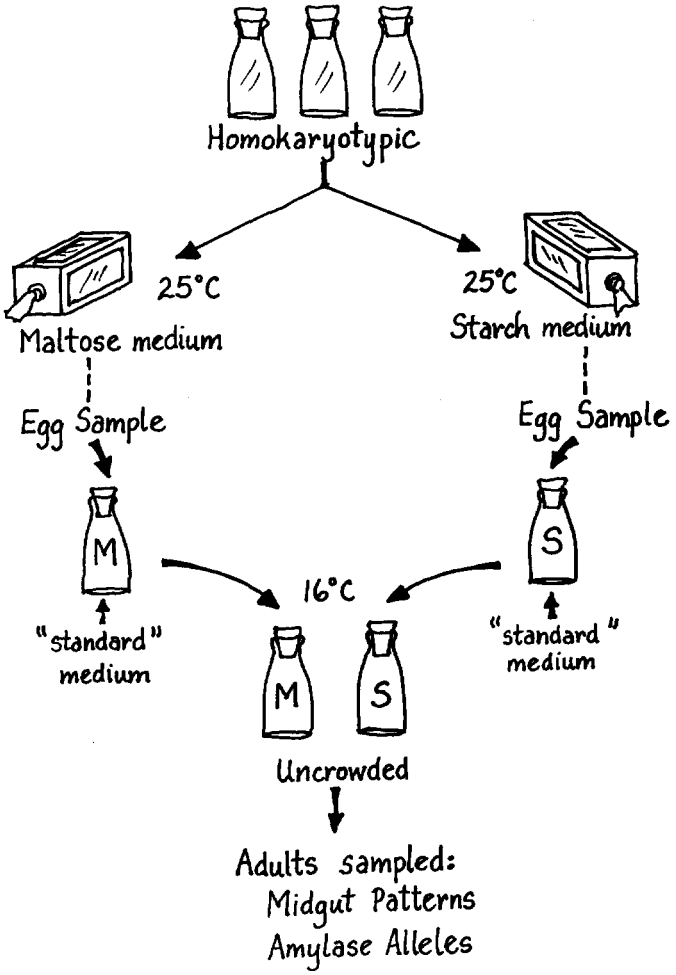

FIGURE 1.—Design of experiment.

and 2. Presence of amylase activity is indicated by a number and absence of detectable activity by a zero. Thus, pattern 100-00 would have activity only in the most anterior region of the AMG.

RESULTS

Starting populations: The conditions under which these populations were maintained were clearly stressful for *D. pseudoobscura*. Twenty-five degrees centigrade is near the temperature maximum for continuous breeding, and the starch and maltose media are not as suitable as standard medium which has a mixture of several carbohydrates. These conditions caused difficulty in starting the populations. At first many populations died within a month, and we had to restart them. Finally, we found that, if the starting cages were kept at 16° for 7 to 10 days before moving to 25°, virtually all populations survived. Thus, the first generation appeared to be particularly stressed but, if a population could

survive the initial stress, continuous breeding of large numbers was established. Although 500 or more individuals were used to start each population, it is likely that this initial stress resulted in a lower number of actual founders of each population.

Generation time: Another indication of the stressfulness of the media was the generation time. After initiating a population it was about 40 days before newly eclosed adults appeared. Therefore, initially, we thought that the generation time was about 40 days. After populations were established for about 2 years, we tested egg to adult development time on the starch and maltose media. Then, we found it to be approximately 15 days, more or less the "normal" rate on standard medium at 25°. This is an indication that the populations have adapted over time to the stressful conditions. This observation also adds ambiguity to the number of generations elapsed at any given time. (In retrospect, it is possible that we overestimated the original generation time due to the fact that females may have been reluctant to lay eggs when first exposed to the novel media. However, the experiment described in the following paragraph argues against this explanation.)

Carbohydrate utilization: To ascertain whether the flies are utilizing the carbohydrates, starch or maltose, the following experiment was done. Three sets of bottles, each containing 50 ml of medium were prepared. One set had the starch medium, one set the maltose medium, and one set had the same medium without starch or maltose. A set number of same aged adults was placed in each bottle. After 48 hours estimates of the density of eggs laid on each type of medium were made; there were no significant differences among media. After 10 days the adults were removed and the resulting F_1 adults counted. The results are in Table 1. As can be seen, *D. pseudoobscura* can survive on the no carbohydrate medium, but having starch or maltose produces about 40 times more adults. This is good evidence that the starch and maltose are serving as important resources.

F_2 test: As can be seen in Figure 1, the sample used to estimate the population frequencies are F_1 of adults living on the two different media. It is possible that the developmental patterns of these F_1 were affected in the egg stage by mothers experiencing different environments. To test for this we reared four samples (two maltose and two starch) to a second generation removed; the same conditions were used, *i.e.*, uncrowded, standard medium, 16°. We then sampled the F_2 for midgut patterns of amylase activity and compared them with their parents. In no case were statistically significant differences found. This confirms that the changes in midgut pattern frequencies are genetic changes.

Experiment I: In experiment I, the frequencies of midgut patterns and *Amy* alleles were determined in eight isofemale lines thought to be homozygous AR. These were mixed in unequal proportions in order to start populations at different frequencies. In each case half of a mixture was placed in a maltose cage and half in a starch cage. Subsequently, two cages were found to be segregating for the ST third chromosome and, therefore, the data for these two are disregarded. (We do not know whether the presence of ST was due to inadequate screening or contamination.) The designation of a population is a

TABLE 1

Mean number (± standard deviation) of adults emerging from a half pint bottle with 50 ml of three types of media

Starch	Maltose	No carbohydrate
78.2 ± 23.4	85.8 ± 30.5	2.2 ± 0.5

TABLE 2

Frequency of Amy^S *in six populations of experiment I*

Days	A^{ST}	B^{ST}	D^{ST}	B^{MA}	C^{MA}	D^{MA}
0	0.02	0.30	0.07	0.30	0.09	0.07
40	0.14	0.23	0.11	0.47	0.34	0.21
80	0.24	0.32	0.14	0.32	0.49	0.09
120	0.15	0.32	0.10	0.43	0.25	0.16
160	0.18	0.08	0.10	0.41	0.19	0.21
200	0.15	0.15	0.12	0.31	0.16	0.24
240	0.22	0.11	0.04			
565	0.33	0	0	0.41	0.46	0.55
730	0.21	Lost	0	0.28	0.51	0.34

The frequency at the start (day 0) is estimated (see text). The sample size (no. of genes) at subsequent times is, with rare exception, 96 per population.

capital letter with a superscript ST for starch and MA for maltose. Cages with the same letter are exact replicas of one another (see MATERIALS AND METHODS). The starch replica of C and the maltose replica of A were the ones found segregating for ST. Results from the remaining six are in Tables 2 and 3 and Figures 2 and 3.

There were only two alleles segregating at the *Amy* locus in these populations; they are designated Amy^S and Amy^F and are probably PRAKASH and LEWONTIN'S (1968) $Amy^{0.84}$ and $Amy^{1.00}$, respectively. As can be seen in Table 2 and Figure 2, for the first several samples there were no clear-cut trends apparent, although Amy^S did seem to be in higher frequency in maltose cages. By 560 days the divergence is clearer: two of three starch populations, B^{ST} and D^{ST}, had gone to fixation of Amy^F; the three maltose populations all had a higher frequency of Amy^S than the one starch cage still segregating for Amy^S.

The divergence of the frequencies of midgut patterns is more dramatic (Table 3 and Figure 3). The most remarkable change is the great increase in one pattern, 100-00, in all three starch populations. In general, this increase appears to occur at the expense of patterns with activity in the PMG (the right half of Table 3). In the maltose populations no consistent pattern of change is evident.

Experiment II: As mentioned previously, experiment II was begun somewhat differently from experiment I. First, all populations were begun with the same mixture of strains so starting frequencies are the same. Second, the starting frequencies were not estimated but determined directly on the starting population: 128 individuals were electrophoresed and 122 dissected to determine midgut activity pattern. Cage designations are as in experiment I except Roman

TABLE 3

Frequencies of midgut amylase activity patterns in experiment I

		Pattern									
Population	Day	100-00	120-00	103-00	123-00	100-10	120-10	103-10	123-10	Other	*n*
A^{ST}	0	0.04	0.17	0.12	0.20	0.03	0.25	0.01	0.10	0.09	
	40	0.59	0.28	0.09	0.04						54
	80	0.50	0.24	0.11	0.11					0.03	62
	120	0.35	0.13		0.02	0.22	0.17		0.11		63
	160	0.29	0.21	0.17	0.08	0.08	0.02	0.02	0.14		63
	200	0.66	0.23	0.03	0.03	0.05					65
	240	0.67	0.25		0.05	0.02	0.02				63
	565	0.74	0.08	0.11		0.02	0.02	0.03			61
	730	0.57	0.25	0.08	0.03		0.03	0.03			63
B^{ST}	0	0.30	0.07	0.01	0.18	0.13	0.17	0.05	0.07	0.01	
	40	0.78	0.22								51
	80	0.84	0.11	0.02			0.02	0.02			65
	120	0.31	0.26	0.08	0.07	0.02	0.08		0.16	0.02	61
	160	0.45	0.28	0.16	0.08	0.02	0.02				64
	200	0.73	0.11	0.06	0.05	0.04	0.02				63
	240	0.60	0.29	0.02		0.05	0.02		0.03		63
	565	0.87	0.04			0.09					63
	Lost										
D^{ST}	0	0.21	0.06	0.19		0.21	0.32		0.01		
	40	0.63	0.25	0.07		0.05					59
	80	0.86	0.02	0.03	0.03	0.03	0.02		0.02		64
	120	0.55	0.14	0.11	0.05	0.06	0.05		0.03	0.02	64
	160	0.60	0.14	0.14	0.08		0.02	0.02	0.02		65
	200	0.43	0.34	0.10	0.04		0.04		0.03		67
	240	0.46	0.29	0.11	0.09	0.02	0.02		0.02		65
	565	0.85	0.03			0.12					60
	730	0.75	0.11	0.09	0.02	0.02	0.02				63
B^{MA}	0	0.30	0.17	0.01	0.18	0.13	0.17	0.05	0.07	0.01	
	40	0.36	0.32	0.03	0.03	0.02	0.22		0.05	0.02	59
	80	0.27	0.34	0.03	0.14	0.03	0.07	0.03	0.08		59
	120	0.39	0.34	0.03	0.03		0.12		0.06		65
	160	0.28	0.35	0.17	0.14		0.02	0.02	0.03		65
	200	0.27	0.25	0.16	0.14	0.02	0.03	0.06	0.06		64
	565	0.31	0.15	0.06	0.05	0.26	0.12	0.05			65
	730	0.18	0.13	0.18	0.05	0.11	0.19	0.08			62
C^{MA}	0	0.15	0.16	0.08		0.18	0.27	0.06	0.06	0.03	
	40	0.19	0.24		0.09	0.10	0.26		0.12		58
	80	0.17	0.33	0.10	0.14	0.03	0.06		0.14	0.02	63
	120	0.22	0.14	0.05	0.05	0.19	0.19	0.03	0.13	0.02	64
	160	0.32	0.34	0.08	0.05	0.11	0.06	0.03	0.02		65
	200	0.34	0.31	0.05	0.08	0.11	0.05	0.02		0.06	65
	565	0.43	0.25	0.06	0.05	0.06	0.08	0.02	0.05		63
	730	0.38	0.11	0.13	0.06	0.16	0.08	0.03	0.05		63

TABLE 3—Continued

Population	Day	Pattern									
		100-00	120-00	103-00	123-00	100-10	120-10	103-10	123-10	Other	*n*
D^{MA}	0	0.21	0.06	0.19		0.21	0.32		0.01	0.01	
	40	0.22	0.15		0.07	0.08	0.18	0.03	0.25		60
	80	0.16	0.28	0.14	0.16	0.03	0.08	0.02	0.14		64
	120	0.44	0.22	0.11	0.16	0.03	0.08	0.02	0.14		64
	160	0.42	0.28	0.14	0.02	0.14		0.02			65
	200	0.38	0.35	0.14	0.06	0.03	0.03				65
	565	0.24	0.32	0.10	0.03	0.15	0.11	0.03	0.02		62
	730	0.23	0.31	0.15	0.11	0.08	0.11		0.02		62

Starting frequencies (day 0) were estimated (see text). Nomenclature of pattern designations follows POWELL and LICHTENFELS (1979) and DOANE (1980).

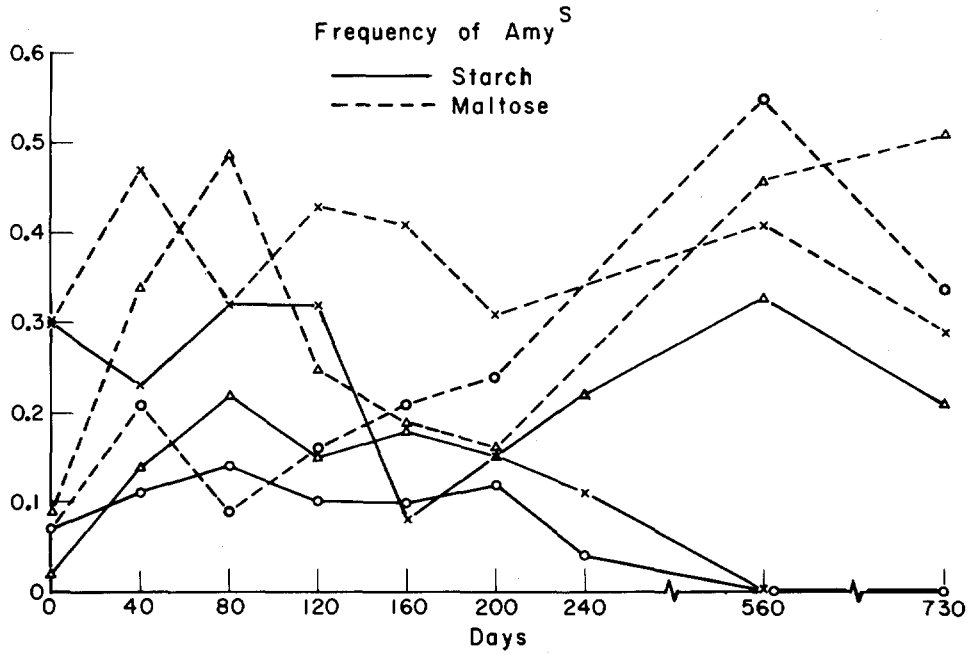

FIGURE 2.—Changes in Amy^S frequencies in experiment I. X = B cages; ○ = D cages; △ = A^{ST} or C^{MA}.

numerals are used instead of letters. The results from experiment II are in Tables 4 and 5 and Figures 4 and 5. Again, pattern 100-00 increased in all four starch replicas, whereas no particularly consistent changes occurred in the maltose cages. Thus, the results are quite consistent with experiment I, although they differ in some details. For example, the divergence in *Amy* alleles became obvious sooner in experiment II. Also, in experiment I there appeared to be an actual increase in Amy^S in maltose cages, whereas no increase is yet apparent in experiment II.

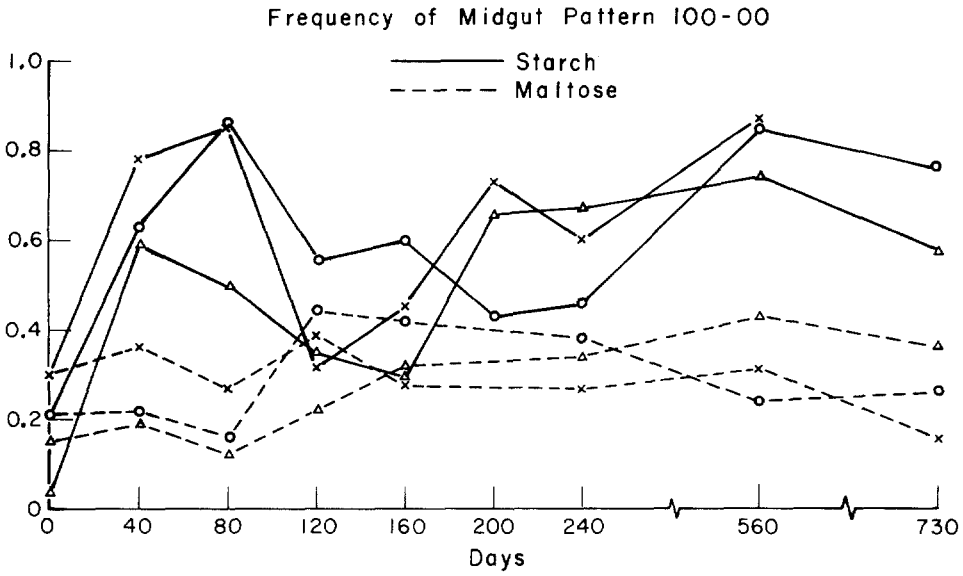

FIGURE 3.—Changes in midgut activity pattern 100-00 in experiment I. Symbols as in Figure 2.

TABLE 4

Frequency of Amy^S *in eight populations of experiment II*

Days	I^{ST}	II^{ST}	III^{ST}	IV^{ST}	I^{MA}	II^{MA}	III^{MA}	IV^{MA}
40	0.03	0.04	0.07	0.04	0.21	0.18	0.27	0.14
80	0.02	0.15	0.06	0	0.19	0.31	0.16	0.31
120	0	0	0	0.10	0.15	0.16	0.21	0.13
200	0	0	0.02	0.05	0.15	0.17	0.13	0.36
276	0.03	0	0.02	0.05	0.09	0.19	0.16	0.20
634	0.03	0	0.05	0.17	0.24	0.25	0.17	0.25

The frequency at the start (day 0) was the same in all populations: 0.61 ($n = 256$). The sample size (no. of genes) at subsequent times is, with rare exception, 96 per population.

In summary, we observed 14 populations, seven on starch and seven on maltose, diverge genetically in at least two aspects of the amylase system: the frequency of alleles at the structural locus and the pattern in which the gene is expressed in the adult midgut. The divergence is quite consistent across replicas: Amy^F appears favored in starch cages and Amy^S favored (experiment I) or at least not selected against (experiment II) in maltose cages; one pattern of midgut expression, 100-00, is favored in starch cages, whereas little change occurs in midgut pattern frequencies in maltose cages. The rapidity of divergence, especially in frequencies of midgut patterns, is quite dramatic. As noted, there is considerable selection in the first generation or two when the populations are first put on the new media. It is quite likely that a moderate bottleneck occurs early on. Thus, there is a greater opportunity for large genetic changes in early generations than there would be if the populations were always large.

Quantitative assays: We have also done quantitative assays for total amylase activity in crude homogenates of flies derived in the same manner as were the

other samples (Figure 1). The samples were from experiment I after it had been running about 2 years and experiment II after 18 months. Assays were done as described by DOANE (1969) using the DNSA method with minor modifications. Enzyme activity is measured as micromoles of maltose produced times 10^4 per

TABLE 5

Frequencies of midgut amylase activity patterns in experiment II

		Pattern									
Population	Day	100-00	120-00	103-00	123-00	100-10	120-10	103-10	123-10	Other	*n*
A11	0	0.23	0.19	0.31	0.17	0.02	0.03	0.02			122
I^{ST}	40	0.55	0.14	0.14		0.03	0.09	0.02	0.03		65
	80	0.53	0.21	0.08	0.02	0.06	0.05	0.05	0.02		66
	120	0.77	0.05	0.05	0.02	0.10	0.02				60
	200	0.54	0.19	0.24	0.02						63
	276	0.53	0.11	0.11	0.11	0.05	0.03	0.02	0.02		63
	634	0.62	0.12	0.06	0.03	0.08		0.05			65
II^{ST}	40	0.67	0.09	0.13		0.06	0.05				64
	80	0.57	0.22	0.02	0.11	0.05		0.02	0.02		63
	120	0.70	0.11	0.11		0.02	0.03	0.03			64
	200	0.69	0.13	0.11	0.03	0.02	0.02				62
	276	0.68	0.16	0.13	0.02	0.02					62
	634	0.69	0.14	0.09					0.08		65
III^{ST}	40	0.50	0.27	0.03	0.03	0.05	0.08	0.02	0.02		64
	80	0.46	0.24	0.14	0.05	0.06	0.02	0.03			63
	120	0.60	0.19	0.06	0.03	0.03	0.06	0.02			62
	200	0.50	0.16	0.08	0.13	0.05	0.02	0.03	0.03		62
	276	0.59	0.16	0.07	0.10	0.02	0.03	0.02	0.02		63
	634	0.73	0.19	0.04				0.03			67
IV^{ST}	40	0.38	0.34	0.11	0.05	0.05	0.06	0.02			65
	80	0.50	0.15	0.05	0.05	0.08	0.08	0.02	0.05	0.02	60
	120	0.59	0.21	0.08	0.02	0.05	0.02	0.02	0.02		61
	200	0.68	0.15	0.15		0.02					65
	276	0.48	0.26	0.23		0.02			0.02		66
	634	0.86	0.08	0.06							64
I^{MA}	40	0.16	0.25	0.34	0.17		0.05	0.02	0.02	0.02	64
	80	0.42	0.25	0.13	0.11		0.03		0.06		64
	120	0.33	0.16	0.14	0.10	0.06	0.14	0.05	0.02		63
	200	0.18	0.32	0.17	0.26		0.03	0.02	0.02		65
	276	0.27	0.27	0.16	0.06	0.03	0.14	0.03	0.05		64
	634	0.26	0.22	0.15	0.14	0.04	0.05	0.09	0.08		65
II^{MA}	40	0.18	0.33	0.21	0.10		0.10	0.03	0.05		62
	80	0.34	0.24	0.16	0.05	0.10	0.07	0.03			58
	120	0.31	0.20	0.20		0.17	0.08	0.05			62
	200	0.17	0.37	0.16	0.17	0.03	0.03	0.03	0.03		63
	276	0.28	0.26	0.19	0.13	0.04	0.04	0.04	0.01		69
	634	0.24	0.21	0.17	0.11	0.05	0.05	0.08	0.09		63

TABLE 5—Continued

Population	Day	Pattern									
		100-00	120-00	103-00	123-00	100-10	120-10	103-10	123-10	Other	n
III^{MA}	40	0.22	0.23	0.22	0.18	0.02	0.02	0.06	0.06		65
	80	0.24	0.23	0.16	0.11	0.09	0.03	0.03	0.09		70
	120	0.31	0.36	0.15	0.11		0.03	0.02	0.02		61
	200	0.28	0.35	0.26	0.06	0.03	0.02				65
	276	0.28	0.30	0.15	0.08	0.05	0.03	0.03	0.03		60
	634	0.29	0.22	0.12	0.13	0.09	0.03	0.01	0.09	0.01	68
IV^{MA}	40	0.30	0.29	0.14	0.06	0.08	0.08		0.05		63
	80	0.19	0.24	0.13	0.11	0.05	0.18	0.03	0.06		62
	120	0.38	0.17	0.14	0.05	0.08	0.09	0.06	0.03		65
	200	0.33	0.19	0.16	0.14	0.05	0.08	0.03	0.03		64
	276	0.21	0.23	0.18	0.06	0.10	0.10	0.05	0.08		62
	634	0.20	0.17	0.09	0.14	0.09	0.09	0.09	0.08	0.05	65

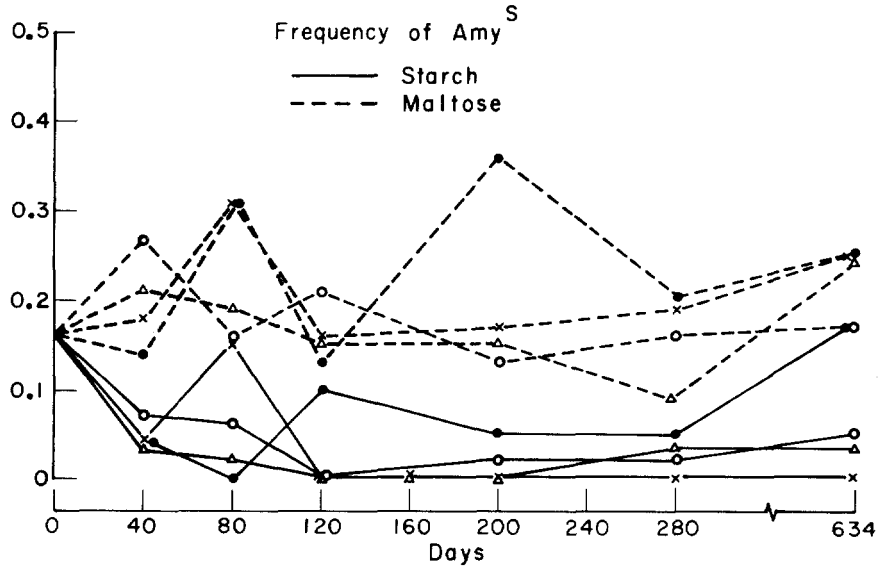

FIGURE 4.—Changes in *Amy*^{S} frequencies in experiment II. △ = I cages; X = II cages; ○ = III cages, ● = IV cages.

minute per micrograms of protein at 25°, pH 7.4 (DOANE 1969). The means of two separate assays, each with two replicas, are in Table 6. Although there is a slight tendency for flies derived from starch cages to have higher activity, it is not very great nor consistent. The mean activity (±1 S.E.) across all seven starch cages for females is 3.02 ± 0.19 and for females from maltose cages 2.71 ± 0.36. The same figures for males are 2.48 ± 0.25 and 2.22 ± 0.33, respectively.

DISCUSSION

Considering the consistency of the results across seven replicas with each type of medium, there is little doubt that adaptive genetic divergence has

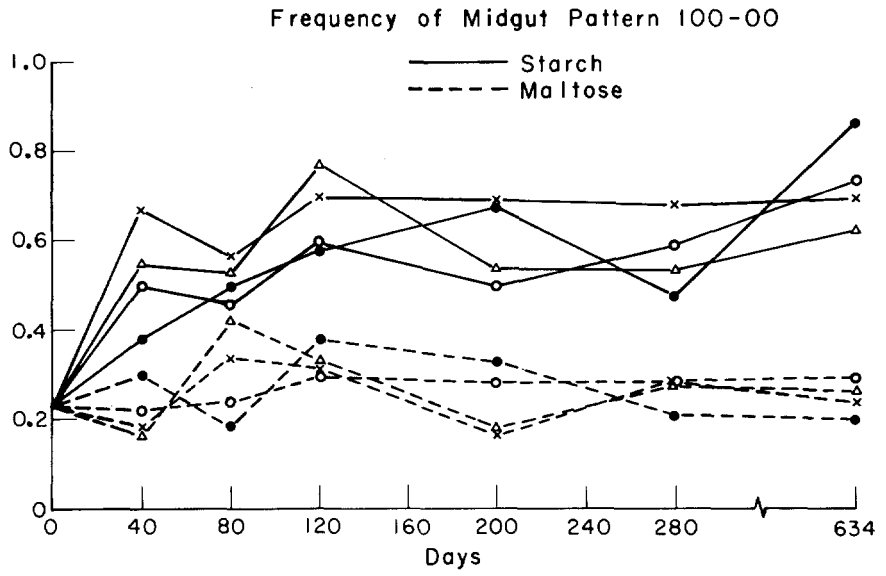

FIGURE 5.—Changes in midgut activity pattern 100-00 in experiment II. Symbols as in Figure 4.

TABLE 6

Amylase activity in 3- to 5-day-old adult D. pseudoobscura *from seven experimental cages*

Cage	Starch	Maltose
A	2.80	
	2.82	
B	3.68	3.73
	3.11	2.88
C		3.37
		2.67
D	3.35	3.62
	2.70	3.61
I	3.15	3.62
	1.79	1.96
II	3.24	1.52
	3.22	1.25
III	2.08	1.66
	1.47	1.22
IV	2.87	2.15
	2.25	1.93

Activity is in maltose units produced per microgram of protein (DOANE 1969). Upper figure is for females, lower for males. Figures are means for four assays; standard errors range from 0.05 to 0.16.

occurred in these Drosophila populations in response to carbohydrates and that α-amylase is very likely involved. That the populations are differentially adapted to carbohydrates has been confirmed by direct viability tests of flies from different cages reared on the two media (D. DODD, unpublished observations). We believe the design of the experiments reported here was successful in detecting selection for at least two reasons: (1) an enzyme was chosen that directly interfaces with the environment, *i.e.*, the substrate of the enzyme originates externally; and (2) because of this, we could manipulate the environment so the limiting resource (Table 1) was either the substrate or product of the enzyme; thus, there is a positive control. By choosing such a design, we circumvent some of the problems inherent in such studies and maximize the probability of detecting selection. For example, KACSER and BURNS (1981) showed that in large metabolic pathways, the function of any single enzyme can vary considerably with only slight effect on metabolic flux. By choosing the first enzyme in a catabolic pathway and by experimentally changing the level of the substrate, much smaller functional differences in allozymes should be detectable on a physiological level and, therefore, of potential adaptive significance.

Is it possible that the "true" targets of selection were neither *Amy* nor the genes controlling its expression but rather some closely linked genes? For several reasons we feel this is unlikely. First, we attempted to randomize the genetic background by starting the cages with many (minimum of 32) independently derived genomes. Second, nearly identical results were observed with populations begun by independent samples collected about 2 years apart. Third, why should there be closely linked genes that respond to the presence or absence of starch? To be sure, changes at gene loci other than those affecting amylase have probably occurred in these populations. However, it seems improbable that these other loci were both tightly linked and in linkage disequilibrium with the genes we monitored.

Previous studies on *D. pseudoobscura Amy* allozymes in laboratory populations have all been done with standard cornmeal-molasses medium and have yielded ambiguous results. YARDLEY, ANDERSON and SCHAFFER (1977) interpreted their results as being consistent with neutrality, whereas WILSON'S (1980) reanalysis of this data indicated nonrandom changes in allozyme frequencies. ANDERSON, SALCEDA and TURNER (1979) studied a population polymorphic for third chromosome inversions as well as *Amy* alleles. The frequency changes in *Amy* alleles could not be accounted for solely by hitchhiking on the inversions and, therefore, selection at *Amy* was indicated.

A few previous Drosophila studies have manipulated medium in an attempt to specifically stress one or a few enzyme functions, all of which report positive results, although not always unambiguously. WILLS and NICHOLS (1971) detected heterozygote superiority at the octanol dehydrogenase locus (*Odh*) when flies were stressed on octanol supplemented medium. YAMAZAKI (1972) argued this result may have been due to a general heterotic effect and not specifically *Odh*. BIJLSMA (1978) obtained evidence for selection at two glucose-metabolizing enzymes when a fatty acid (octanoate) was a stressful component of the

medium. Alcohol dehydrogenase (*Adh*) has also been stressed by the addition of ethanol in the medium with positive signs of selection occurring on *Adh* alleles (GIBSON *et al.* 1979; WILSON 1980; CAVENER and CLEGG 1978). In similar experiments MCDONALD *et al.* (1977) presented evidence that *Adh* regulation changes may have occurred in Drosophila adapting to ethanol, although CLARKE *et al.* (1979) have questioned this interpretation. Perhaps the most relevant previous study is that of DEJONG and SCHARLOO (1976) who detected fitness differences among *D. melanogaster Amy* genotypes when reared on starch medium.

In addition to Drosophila such studies have been done with microorganisms such as yeast (e.g., FRANCIS and HANSCHE 1973) and *E. coli* (e.g., DYKHUIZEN and HARTL 1980). This latter report highlights the complexities that may arise: some alleles at the 6-phosphogluconate dehydrogenase locus were clearly subject to selection, some were apparently neutral, whereas still others gave evidence for selection under some conditions but not others.

Why should Amy^F and midgut pattern 100-00 be favored in our starch medium cages? We cannot answer this because we do not know enough about the biochemistry and physiology of α-amylase in Drosophila. The rise in frequency of a midgut pattern with limited midgut activity in starch environment was not anticipated. However, the limited midgut activity in starch cage flies has not been accompanied by a reduction in total specific activity (Table 6). Evidently, there has been a shift in the tissue distribution from the midgut to other tissues, perhaps the hemolymph. It may also be that the adult shift in expression was really caused by selection in the larval stage which carries over to controlling adult expression. In any event, there was a clear-cut change in *Amy* regulation.

One can argue that the results we present were obtained in a contrived laboratory situation and may have little relevance for what is happening in nature. This is true. However, the fact that our experiments, as well as those cited, often give indications of selective differences among allozymes when specifically stressed certainly lends plausability to the selectionist view that enzyme variants are subject to the forces of natural selection. If such studies consistently yielded no evidence for selective differences in the contrived situations, it would be difficult to defend a selectionist interpretation in more "natural" environments. These laboratory results indicate at least a *potential* for selection to act in nature. [We must add a caveat at this point: How often have such laboratory stress experiments been done with negative results (*i.e.*, no indication of selection) and not deemed of sufficient importance to be published?]

It is important to note that both the gene (allozyme) and gene regulation (midgut patterns) polymorphisms responded to selection in these experimental populations. Our previous results led us to believe that these two polymorphisms were evolutionarily independent, *i.e.*, no strong linkage or epistatic interactions. This was based on the observations that there are no significant correlations between frequencies of morphs of the two systems within individuals (POWELL 1979) or across species (POWELL, RICO and ANDJELKOVIĆ 1980). The present

results are not inconsistent with this view. Two polymorphic systems may respond to the same selective challenge and yet be independent. Furthermore, these results indicate that the argument over the importance of structural gene changes *vs.* gene regulation changes in adaptive evolution is not an either/or question. Rather, it is a question of *relative* importance and the *interplay* between the two types of evolutionary change.

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