McInnis, D.O. and G.C.Bewley. Tropical Fruit and Vegetable Research Laboratory, Honolulu, Hawaii USNA. Laboratory directional selection for dispersal rate and α-GPDH enzyme activity in Drosophila melanogaster.

Quantitative genetic variation for various behavioral traits has been uncovered through directional selection experiments in Drosophila, including phototaxis (Hirsch & Boudreau 1958), and spontaneous locomotion (Grant & Mettler 1969). The effect of major loci, however, has only recently been explored. A major gene-enzyme system involved in flight muscle metabolism in Drosophila is α-glycerophosphate dehydrogenase (α-GPDH, E.C. 1.1.1.8). This enzyme is coupled to a mitochondrial oxidase in the α-glycerolphosphate cycle and provides a mechanism for the rapid and constant energy source required for sustained flight. In this study, (1) directional selection was applied to select for high and low dispersal rates in the laboratory and (2) the relationship between dispersal rate and α-GPDH activity level was investigated.

During 1977, studies were initiated to shed some light upon the important question of how observed variation in realized fly dispersion in the laboratory may be partitioned into genetic and environmental variation. These tests involved wild-raised (1st generation) then laboratory-reared flies (2nd generation onward) of D.melanogaster. Directional selection was applied over successive generations upon tested fly populations with the goal of creating a high line of fast-moving flies and a low line of slow-moving flies. Flies were maintained on standard corn meal molasses medium at 25°C except for the relatively brief periods of the experimental runs. The latter were conducted at 2½-3 week intervals with the adult flies in the same generation. The parents of each generation were removed after a week of mating and egg laying. After eclosion, new generation ♀♀ and ♂♂ (10-14 days old) were allowed to mate prior to the actual experimental run involving selection of fast and slow moving flies. Unfortunately, the control lines for the cage and field work were accidentally lost. However, the divergence between simultaneously run lines was controlled.

Heritability under a system of mass selection (i.e., selected flies mated together en masse) was measured by the regression of the response (R) on selection pressure (S). If all females had mated before selection, and no mating occurred after selection, then $h^2=2$ R/S. If some remating and sperm displacement occurred after selection, then $R/S<h^2<2$ R/S. Estimating this range is appropriate for this work since flies were allowed to mate prior to selection.

Population cage experiments. The following design was established for selecting fast and slow movers in the laboratory. The 'experimental field' was a standard plexiglass population cage (46½ cm long X 14-3/4cm wide X 10½cm high) containing 16 openings for vials (3½ cm diameter X 10cm height) in the floor of the cage. The inside chamber was partitioned into 4 equally sized compartments separated by tightly fitted cardboard. The compartments

Table 1. Directional selection for dispersal rate and α-GPDH activity in a population cage with D.melanogaster (1977).

	Generation number						
	1	2	3	4	5	6	7
Selected line	Compartment position (Avg ± SE; sample size = 200)						
HIGH	(2.52±0.15)	2.78±0.12	2.85±0.11	2.85±0.10	2.85±0.11	2.67±0.14	3.35±0.08
LOW		2.31±0.11	1.98±0.12	2.40±0.14	1.72±0.10	1.62±0.09	1.32±0.07
HIGH-LOW DIVERGENCE	0	0.47	0.87	0.45	1.13	1.05	2.03
CUMULATIVE SELECTION	0	3.00	5.53	7.66	10.21	12.08	14.03
Selected line	α-GPDH activity (units/mg live weight; Avg ± SE)						
HIGH	0.050±.003	0.051±.002	0.050±.002	0.054±.003	0.050±.002	0.054±.002	0.050±.002
LOW	0.058±.003	0.055±.002	0.044±.002	0.048±.003	0.044±.003	0.046±.002	0.040±.002
HIGH-LOW DIVERGENCE	-0.008	-0.004	+0.006	+0.006	+0.006	+0.008	+0.010

were arranged in a horizontal line (i.e., 1-2-3-4) at right angles to light entering the lab from the outside. Flies could travel from one compartment to another by walking through one of 8 circular holes (6mm diameter) punched, equally spaced, around the periphery of the dividers. To provide food and moisture, 4 vials of banana-agar medium were placed in the compartment (#4) most distal to the compartment (#1) wherein the flies were released. Twelve empty vials sealed the remaining holes in compartments 1, 2, and 3.

The basic experimental procedure was as follows. One hundred each of locally wild caught ♀♀ and ♂♂ Drosophila melanogaster provided the basis for the first run. These flies were anesthetized briefly with CO_2 gas and while still immobile, placed inside compartment #1 of the experimental cage. The lid was sealed and the flies allowed to revive and move about for approximately 3 to 4 hr after which time the experiment was abruptly stopped. This was accomplished by rapidly CO_2 gassing the flies and aspirating those in each compartment into separate fresh food vials. Flies collected in compartment #4 became the HIGH line parents for the next generation, while flies taken in compartment #1 (the original release site) became the parents for the next LOW line offspring. Such HIGH and LOW lines were maintained in distinct sets of vials for 7 generations and run in separate cages at the time of each selection cycle. Samples were taken for an α-GPDH enzyme activity assay following each experimental run.

α-GPDH enzyme assay. The extraction of α-GPDH for routine assays was performed by the homogenization of adult samples with Dounce glass tissue grinders at a concentration of 20 mg live weight per ml of homogenization buffer which consisted of 0.1M sodium phosphate (pH 7.1) containing 10 nM EDTA and 0.5 nM DTT. Samples were centrifuged for 30 min at 17,000 g at 0°C. The resultant clear supernatant fluid was used as the enzyme source. Each sample consisted of ca. 30 adult flies (evenly divided by sex) and 2 such samples were run and averaged for each tested line. Enzyme activities were monitored in the reverse direction, GP----DHAP, using a Beckman model 25-spectrophotometer with the sample compartment maintained at 20°C. For this reaction, 0.2 ml of properly diluted enzyme was added to a mixture of 0.1M glycine-NaOH (pH 10.0), 4.5 nM NAD^+, and 15 mM GP. Final volumes were 2.5 ml in all assays. A unit of activity is defined as 1 μmole of NAD^+ reduced per minute based upon a molar extinction coefficient for NADH of 6.22×10^3.

Results. The results of directional selection for 6 generations in the population cage are shown in Table 1. The cage data are recorded in terms of the average compartment position attained by HIGH and LOW line flies each generation. The divergence between the lines (HIGH minus LOW) and the cumulative selection pressure are also shown. Movement date in generation 1 are for the original native population prior to selection of HIGH and LOW lines. The 6 generations of selection in the population cage (gens. 1-7) produced an average realized heritability per generation of 0.11 (11%) with a standard error (of the line slope) of 0.027. Divergence and cumulative selection were significantly correlated ($r=0.890$, $p<.01$) and both a sign test for direction (+ or -) of divergence ($p<.05$) and a t-test ($df=6:4.09$, $p<.01$) indicated significant non-zero heritability for dispersal rate averaged over 6 generations. There was no apparent change in the α-GPDH activity of the HIGH line, while the activity of the LOW line declined, largely between gens. 2 and 3. The dispersal rate of the LOW line flies, but not of the HIGH line flies, was significantly correlated with α-GPDH activity ($r=0.850$, $.01<p<.05$).

In conclusion, the evidence indicates that a genetic component exists for small-scale laboratory dispersal ability in D.melanogaster. The available evidence in this study indicates that there is no direct relationship between α-GPDH enzyme activity and fly dispersal rate in the laboratory.

References: Grant, B. & L.E.Mettler 1969, Genetics 62:625; Hirsch, J. & J.C.Boudreau 1958, J.Comp.Physiol.Psychol. 51:647.