Bishop, E. R. and S. J. Shafer. Dowling College, Oakdale, New York. Learning behavior in D. melanogaster larvae. I.

Larval forms of D. melanogaster have been shown to exhibit the capacity to sense and avoid odorants when such odorants are coupled with electric shock (Aceves-Piña and Quinn 1979). Third instar larvae will preferentially select one of two odorant sources through a method of shock reinforcement. The two-part experimental design in which larvae are trained to discriminate opposite odors eliminates odor bias and sensitization as explanations for the results (Aceves-Piña and Quinn 1979). Using specific controls, pseudoconditioning, excitatory states, odor preference, sensitization, habituation, and subjective bias can be eliminated as reasons for experimental outcome (Quinn, Harris and Benzer). The normal behavior of third instar larvae in the presence and absence of odorants can be statistically evaluated. The two odorants methyl hexanoate and amyl acetate, to which third instar larvae give comparable positive chemotactic responses, wer chosen. Learned behavior can therefore be differentiated from the normal larval response.

Table 1. Larval response (in seconds)

	Chi-square analysis	$\overline{X}$ (seconds)	Standard deviation (seconds)
control	P>0.99	378	230
methyl hexanoate	0.9>P>0.8	72	60
amyl acetate	0.7>P>0.5	63	31

Comparison of samples: t-test

	Probability of sameness
control-methyl hexanoate	P<0.001
control-amyl acetate	P<0.001
amyl acetate-methyl hexanoate	between 0.4 and 0.5

References: Aceves-Piña and Quinn 1979, Science 206:93-96; Quinn, Harris and Benzer 1974, Proc. Nat. Acad. Sci. USA 71:708-712.

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It can be shown that larval forms of wild-type Oregon-R D. melanogaster may be conditioned to avoid odorants for which they are normally chemotactically positive when such odorants are associated with electric shock (Aceves-Piña and Quinn 1979). This behavior, termed olfactory learning ability, may be statistically evaluated and the index of learning (the fraction of the population avoiding the shock associated odor minus the fraction avoiding the control odor) may be determined. Values range from -1 to +1. A value of one or the avoidance of the shock-associated odorant in all trials indicates "absolute" learning. A value of zero indicates that the training of larvae with shock does not alter odorant preference. A negative value indicates that the larvae run preferentially to the shock-associated odorant source. The values reported for learning deficient third instar larvae obtained from crosses between males treated with ethyl methanesulfonate and normal females may be compared to the values obtained for normal third instar larvae.

The odorants amyl acetate and methyl hexanoate to which normal third instar larvae exhibit positive chemotactic responses were selected. The two-part design in which larvae are trained to opposite odors eliminates odor bias and sensitization as explanations for the results (Quinn and Aceves-Piña 1979). It can be shown that normal and treated larvae exhibit a marked difference in learning behavior. Using a 2x2 contingency table with one degree of freedom and the competing hypothesis:

H_o: There is no difference in avoidance behavior between larvae trained with amyl acetate associated with electrical shock and larvae trained with methyl hexanoate associated with electrical shock, and

H_1: There is a difference in avoidance behavior between larvae trained with amyl acetate associated with electrical shock and larvae trained with methyl hexanoate associated with electrical shock,

and assuming H_o to be true with a 5% level of significance, H_o shall be accepted if $\chi^2 \leq 3.84$. Based on the results exhibited in Table 1 for normal larvae, H_o shall be accepted. Since normal third instar larvae behave similarly when conditioned with either of the two attractants,

a combined chi-square comparing avoidance to non-avoidance was done. Based on random movement, it was expected one half of the sample will avoid the shock-associated odorant while one half will not. Using a sample size of 60 and the observed avoidance to non-avoidance ratio of 39:21 with one degree of freedom, the P value ($0.025>P>0.010$) obtained indicated the movement obtained was not random and was therefore attributed to olfactory learning with the index of learning for normal larvae equaling 0.30.

A second 2x2 contingency table with one degree of freedom and the competing hypothesis:

H_o: There is no difference in avoidance behavior between larvae from treated lineages trained with amyl acetate associated with electrical shock and larvae from treated lineages trained with methyl hexanoate associated with electrical shock, and

H_1: There is a difference in avoidance behavior between larvae from treated lineages trained with amyl acetate associated with electrical shock and larvae from treated lineages trained with methyl hexanoate associated with electrical shock,

and assuming H_o to be true with a 5% level of significance, H_o shall be accepted if $\chi^2 \leq 3.84$. Based on the results exhibited in Table 1 for treated lineages, the null hypothesis was accepted.

In conclusion, a 2x2 contingency table with one degree of freedom was used to compare the final competing hypotheses:

H_o: Normal third instar larvae and third instar larvae from treated lineages behave similarly in conditioning experiments when either of the two attractants are accompanied with electrical shock, and

H_1: Normal third instar larvae and third instar larvae from treated lineages do not behave similarly in conditioning experiments when either of the two attractants are accompanied with electrical shock,

and assuming H_o to be true with a 5% level of significance, H_o shall be accepted if $\chi^2 \leq 3.84$ (Table 2). Based on the results of Table 2, the null hypothesis was rejected. Third instar larvae from normal and treated lineages did not behave similarly in conditioning experiments when either of the two attractants were accompanied with electrical shock.

A chi-square analysis using a sample size of 60, an observed avoidance to non-avoidance ratio of 26:34, one degree of freedom and the expected avoidance to non-avoidance ratio of 39:21, the resulting P value of less than 0.005 indicated the movement of treated larvae is not the same as normal larvae. The index of learning (larvae from treated lineages) equaled -0.13. Third instar larvae from treated lineages are learning deficient as compared to the index of learning for normal larvae.

Table 1. Sample of 60 larvae.

	Shock associated odorant	Avoidance of odorant	No avoidance of odorant	Total	
normal	amyl acetate	19	11	30	$\chi^2 = 0.07$
	methyl hexanoate	20	10	30	$0.90>P>0.75$
		39	21	60	
treated	amyl acetate	14	16	30	$\chi^2 = 0.27$
	methyl hexanoate	12	18	30	$0.75>P>0.50$
		26	34	60	

Table 2.

Larval type	Avoidance of odorant	No avoidance of odorant	Total	
normal	39	21	60	$\chi^2 = 5.6$
treated	26	34	60	$0.025>P>0.010$
	65	55	120	

References: Aceves-Piña and Quinn 1979, Science 206:93-96.