

References: Astauroff, B.O. 1930, *Z. Indukt. Abstamm. u. Vererbungslehre* 55:183-262; Falconer, D.S. 1981, "Introduction to Quantitative Genetics" Longman Group Ltd.; Reeve, E.C.R. & F.W. Robertson 1954, *Z. Indukt. Abstamm. u. Vererbungslehre* 86:269-288; Suzuki, D.T., A.J.F. Griffiths & R.C. Lewontin 1981, "An Introduction to Genetic Analysis" Freeman & Co.

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 1 Augsburg College, Minneapolis, Minnesota  
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 Honolulu, Hawaii USNA. A new arrival to  
 the Hawaiian Islands: *Drosophila cardini*.

In March of 1983 a female *Drosophila* was collected in Honolulu near Leahi Hospital. The collection site was at the southeast corner of Kilauea Avenue and Makapuu, near property occupied by Kapiolani Community College. The fly was caught in a banana trap hung from branches of what appeared to be the cactus

*Cereus undatus*. This female died without laying any eggs, but her external characters indicated that she was probably a member of the *cardini* group. These characters included: clouded crossveins; black bands on the posterior edge of yellowish-brown abdominal tergites, with the black in the lateral areas not reaching the lateral edge of the tergites and extending anteriorly and medially in the posterior tergites; brilliant orange eyes; and reddish-brown mesonotum, scutellum and pleurae. Another female was collected from the same site in August of 1983, again using a banana bait. This female was successful in laying eggs, and an isofemale line was established. Examination of males of this line showed that they lacked a protuberance on the anteroventral margin of the labellum and possessed anal plates with one or two long anteriorly directed bristles on the anteromedial corner of the plates. These features are characteristic of *D. cardini* Sturtevant (Stalker 1953).

Since the *cardini* group consists of about 16 sibling and near-sibling species, the metaphase chromosome group was determined by brain smears. This showed the presence of 6 pairs of chromosomes, including 5 pairs of acrocentrics and one pair of microchromosomes. *D. cardini* is the only member of the species group which has this somatic metaphase figure (Futch 1962; Heed & Russell 1971). The chromosomal and morphological features thus lead to the conclusion that these flies are members of *Drosophila cardini* Sturtevant. This species has been found in Florida, Mexico, Central and South America, and the West Indies but has never before been recorded from the Hawaiian Islands. In fact no other species of *cardini* group has been found here. *D. cardini* thus represents a new arrival to the Hawaiian Islands.

Acknowledgement: These flies were collected while the first author was on leave at the Arbovirus Program, Pacific Biomedical Research Center, Univ. of Hawaii, Honolulu, USNA HI.

Ref: Futch, D.G. 1962, *Univ. Texas Publ.* 6205:539-554; Heed, W.B. & J.S. Russell 1971, *Univ. Texas Publ.* 7103:91-103; Stalker, H.D. 1953, *Ann. Ent. Soc. Amer.* 56:343-358.

Holliday, M. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois.  
 Excitatory (Pavlovian) Conditioning.

Using a variation of Nelson's (1971) procedure for conditioning the blow fly *Phormia regina*, we demonstrate excitatory conditioning of the proboscis extension reflex in *D. melanogaster*.

The flies came from a population established by crossing the Berlin line with another produced by mixing nine Austin, TX inbred lines obtained from Birmingham, England. They were virgin males and females, 44-48 hr old, 36 hr food deprived and water satiated, when conditioned on the automated stimulating apparatus (Vargo, Holliday & Hirsch 1983; Holliday, Vargo & Hirsch 1983).

As outlined in Figure 1, the conditioning procedure presents for 5 sec to the foretarsi a 0.5-M NaCl conditioned stimulus (CS), followed after a 0.5-sec interval by a 0.25-M sucrose unconditioned stimulus (US) for 5 sec (also accessible to the proboscis for 2-3 sec), itself followed after a 170-sec interval by a distilled H<sub>2</sub>O intertrial stimulus (ITS) for 5 sec, which, in turn, is followed after a 175-sec interval by the start of the next trial. Thus, the intertrial interval (ITI) is 6 min. The ITS serves to discharge any residual sucrose induced excitatory state (CES, Dethier, Solomon & Turner 1965) which in *D. melanogaster* can last at least 10 min (Vargo & Hirsch 1982a, 1982b). With a 6-min ITI, it is important to discharge CES in order to avoid confounding non-associative excitation with associative responding (conditioning) to the CS.

For 111 flies Figure 2 presents average results combined from four experiments. Over nine trials, responding (1) to the CS increases significantly (regression coefficient:  $B=3.7$ ,

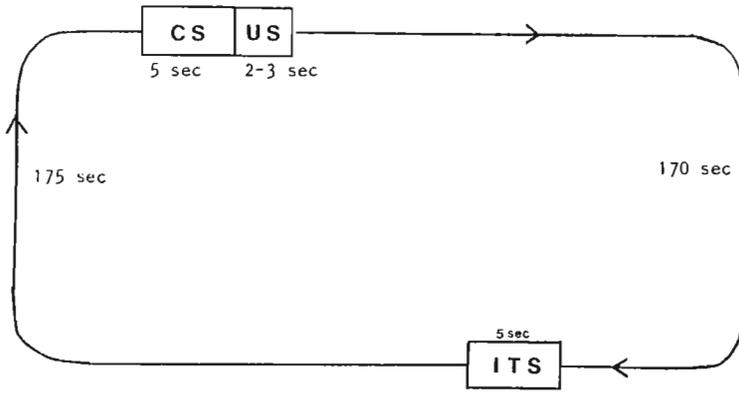


Fig. 1. Conditioning stimulus schedule.

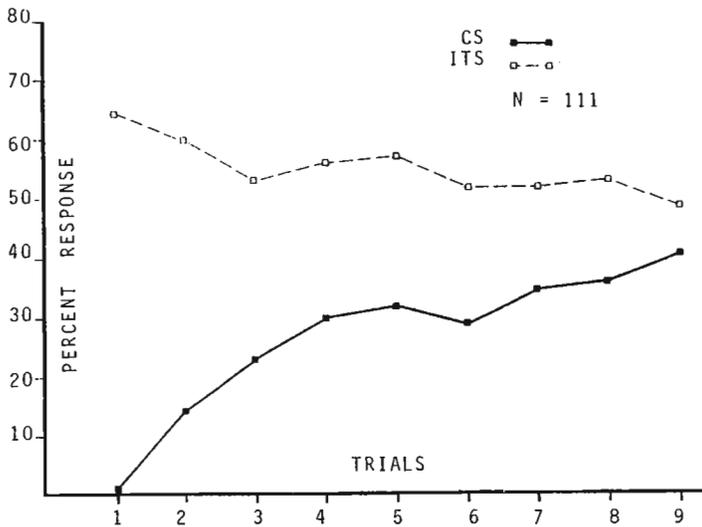


Fig. 2. Conditioning: Percent response over trials to CS and ITS for 111 flies (including the 22 flies from the paired group in Figure 3).

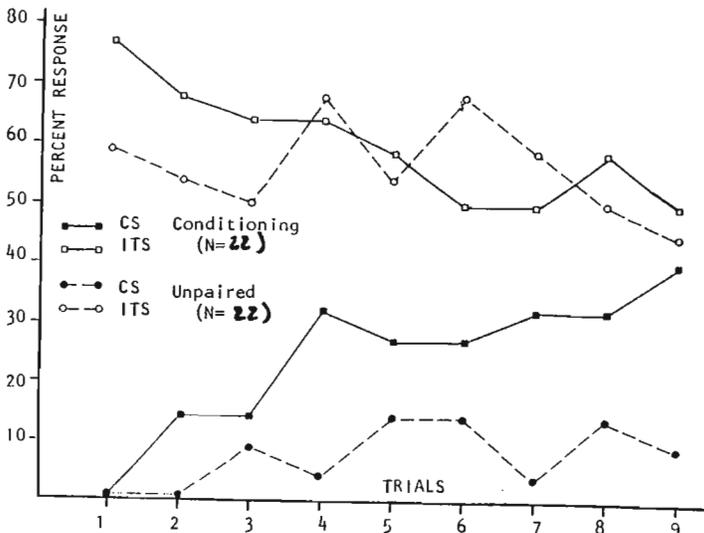


Figure 3. Percent response over trials to CS and ITS for an unpaired control and a paired conditioning group. (N = 22 per group).

$p < 0.05$ ), thus showing conditioning, but (2) to the ITS decreases significantly though less steeply ( $B = 1.58$ ,  $p < 0.05$ ).

To demonstrate the effectiveness of the ITS for discharging CES, a control group was tested on the same day as a conditioning group--an unpaired CS was positioned to precede the US by 90 sec on the assumption that such a separation would prevent conditioning, which requires CS-US contiguity. Figure 3 shows the effect of any residual post-ITS CES on the conditioned response to be minimal. Over trials, the unpaired group ( $N=22$ ) shows a much smaller increase in responding than does the paired (conditioning) group ( $N=22$ ), with a significant difference between the two (Kolmogorov-Smirnov two-sample test, two-tailed probability:  $Z=1.41$ ,  $p < 0.037$ ). Additional control experiments have demonstrated that neither sensitization nor pseudoconditioning has a significant influence on the conditioned response.

This unique procedure permits train/testing 18 individuals in a 54-min session and yields a complete response record of the conditioning of each individual.

References: Dethier, V.B., R.L. Solomon & L.H. Turner 1965, *J. Comp. & Physiol. Psych.* 60:303-313; Holliday, M., M. Vargo & J. Hirsch 1983, *DIS* 59: 140-141; Nelson, M.C. 1971, *J. Comp. & Physiol. Psych.* 77:353-368; Vargo, M. M. Holliday & J. Hirsch 1983, *Behav. Res. Meth & Instrumen.* 15:(1)1-4; Vargo, M. & J. Hirsch 1982, *J. Comp. & Physiol. Psych.* 96:452-459.

Acknowledgement: This work was supported by a Grant for research training in Institutional Racism (MH15173), from the National Institute of Mental Health; a Biomedical Research Support Grant (NIH RR 7030-1-5-21332), from the National Institute of Health; a Grant for research in Behavior-Genetic Analyses (BNS-83-00353), from the National Science Foundation.

Kambysellis, M.P., P.Hatzopoulos, and E.M. Craddock. New York University (New York) and State University of New York (Purchase) New York USA. Rapid in vivo incorporation of radioactive amino acids into vitellogenin proteins of *Drosophila grimshawi*.

In our studies on the timing of vitellogenin protein synthesis in *D.grimshawi* (Kambysellis, Hatzopoulos & Craddock 1984), we have found that by feeding the flies for a brief time (1-5 minutes) with radioactive amino acids, sufficient label is introduced into the flies to permit in vivo incorporation into proteins for several days. In representative experiments

outlined here, groups of five mature 18 day old females were starved for 15 minutes, and then introduced into a feeding chamber which consisted of a plastic shell vial (10 cm long, 3.5 cm in diameter, Connecticut Valley Biological Supply Co., Inc.) into which was inserted a piece of Kimwipe paper (2x1.5cm) four layers thick. This paper was saturated with 100  $\mu$ l of a 20% sucrose solution containing a mixture of radioactive amino acids, namely 25  $\mu$ Ci each of  $^3$ H-aspartic acid (s.a. 10.0 Ci/mmol),  $^3$ H-serine (s.a. 16.8 Ci/mmol),  $^3$ H-glycine (s.a. 15.0 Ci/mmol),  $^3$ H-lysine (s.a. 68.0 Ci/mmol) and  $^3$ H-leucine (115.2 Ci/mmol), and 100  $\mu$ Ci  $^{35}$ S-methionine (999.8 Ci/mmol). The paper was then placed in the middle of the vial which was lain on its side as is usual for culturing the large Hawaiian *Drosophila*. Once introduced into the vial, the flies are attracted immediately to the sucrose-amino acid mixture and feed

continuously for about one to one and a half minutes. The same feeding chamber can be used to feed up to ten groups of flies in each experiment. Following feeding (pulse), the flies were placed either on regular Hawaiian *Drosophila* medium (Wheeler & Clayton 1965) or in empty vials for the chase period. At the end of variable periods of chase, the groups of five flies were anaesthetized, their hemolymph collected (Kambysellis 1984), and the fat bodies and ovaries dissected, homogenized in 50  $\mu$ l of 50 mM Tris-HCl pH 8.2, 0.25M NaCl buffer, and centrifuged for one minute in an Eppendorf centrifuge. The aqueous phase

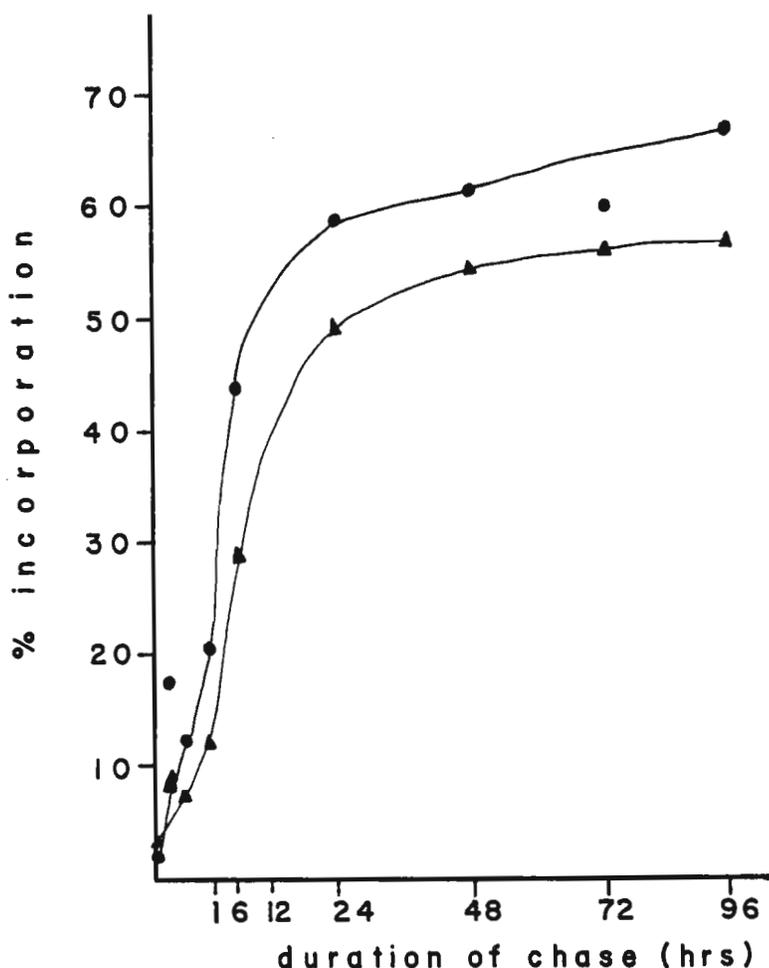


Fig. 1. Incorporation of radioactive amino acids into TCA insoluble proteins. Percentage of incorporation was determined by dividing the number of TCA precipitable counts in a particular sample by the total cpm in that same sample. Notice that the scale on the horizontal axis has been expanded for the first hour of the chase to disclose the four observations during that period.