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Bidirectional selection for central excitation.

A food-deprived, water-satiated fly shows an increased frequency of the proboscis extension reflex (PER) to a water stimulus which follows sucrose stimulation of the labellum but not to a water stimulus which precedes sucrose stimulation. This sucrose induced responsiveness is termed the central excitatory state (CES) with previous studies performed with the blow fly Phormia regina (Dethier et al. 1965,1968). Bidirectional selection experiments for high and low expression of CES in Phormia (McGuire 1981; Tully & Hirsch 1982a) and hybrid analyses of the selected lines (Tully & Hirsch 1982b) have found two segregating alleles of one major gene correlate of CES. Further behavioral experiments revealed the existence of additional components of the proboscis extension reflex (PER; Tully & Hirsch 1983) and that CES was positively correlated with excitatory conditioning of PER in Phormia (Tully et al. 1982).

CES has since been demonstrated in Drosophila melanogaster (Vargo & Hirsch 1982a,b). Furthermore, from other studies there is reason to believe that CES is involved with the summation of courtship stimuli in female D.melanogaster (Bennet-Clark et al. 1973). It would now be valuable to have selected lines of D.melanogaster with extreme expression of CES so that more detailed studies with other behaviors can be performed. The importance of these lines lies in the ability to use CES as a controlled variable in studies of other behavioral constructs which may have CES as a correlated trait.

Two foundation populations of Drosophila melanogaster were used; the Berlin wild type strain obtained from Marburg, Germany in 1975 and an outbred population called Austin, produced by the interbreeding of 12 Austin inbred lines obtained from Birmingham, England. All stocks were kept on a 16/8 hr L/D cycle at 25°C and 50% RH and maintained on Instant Drosophila medium (Formula 4-24, Carolina Biological Supply Co., Burlington, NC).

The basic test procedure was the same as that used in Vargo & Hirsch (1982a,b). Each animal received in a single trial (a) a 5-sec stimulation of the tarsi, and labellum if the proboscis was extended, with distilled water (pretest), (b) a 5-sec stimulation of the tarsi and labellum with .25 M sucrose immediately following pretest, (c) a 45-sec inter-stimulus interval (ISI) immediately following sucrose stimulation in which no stimuli were administered, and (d) a 5-sec presentation of distilled water (posttest) again applied to the tarsi first and labellum if extended. Animals were allowed to imbibe water on pretest and posttest to control for thirst and labellar contact with sucrose was required for a response to be recorded. All animals were approximately 48 hr old at the time of testing and food-deprived by placing them for 36 hr in a vial containing water-soaked cotton. Before a test session all subjects were given water 15 min ad lib to ensure water satiation.

The CES test consisted of 8 trials with a 6-min inter-trial interval (ITI). Proboscis extension was scored all or none (Position 3 or better on the Dethier et al. scale, 1965), therefore the range of scores for pretest, sucrose, and posttest was 0 to 8, with posttest being the measure of CES in an animal.

Animals were stimulated automatically using the apparatus described in Vargo et al. (1983) and Holliday et al. (1983). In the automatic method, the solutions were contained on Whatman #3 filter paper strips which were placed on the surface of a kymograph drum. The animals were positioned along the side of the kymograph. With the drum turning, as the strips approached the fly, it extended its tarsi and walked over the strips, thereby being stimulated. Animals were discarded from analysis if (a) sucrose score was less than 6 or (b) the animals did not participate (not walking over either the pretest, sucrose, or posttest strips) 3 or more trials.

Animals selected to breed the high line were required to score 6 or more on posttest and 2 or less on pretest, whereas to qualify for the low CES line, flies had to score 2 or less on both pretest and postttest. In each generation 4 pairs were mated. A control line was also maintained in which 4 pairs of untested flies were bred each generation. In each generation, equal numbers of males and females were tested. Approximately 30 flies were tested each generation with N ranging from 10 to 50. Four bidirectional selection experiments were performed, three with Berlin and one with Austin. From the four experiments only one high line (termed HE) and one low line (termed LE) were obtained (Figure 1). HE was founded out of Berlin while LE was founded out of Austin. It is interesting to note that from the other selection experiments not presented, Berlin never produced a low line and Austin never produced a high line. Given the above result, the most appropriate control line with which to compare HE and LE would be a hybrid between the Berlin and Austin foundation populations. Such a population was mated. The Berlin, Austin, and hybrid of the two were all tested for

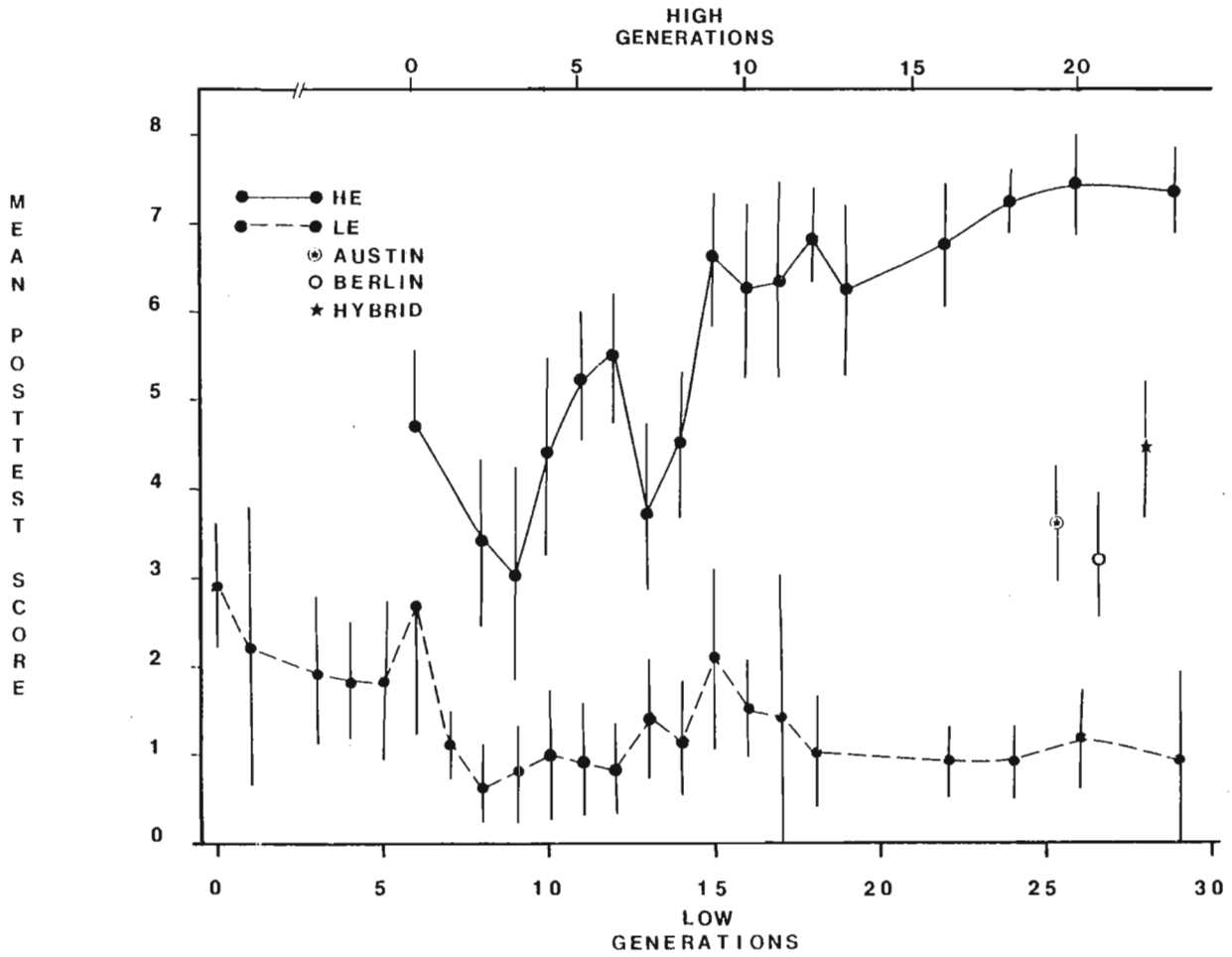

Figure 1. Selection data for the high and low CES lines. The high line was first tested at the 7th generation of the Austin attempt. Plots are presented in this manner to indicate which generations were tested concurrently. Data points for unselected populations of Austin, Berlin, and the Austin X Berlin population are likewise shown to demonstrate the effect of selection and displaced to later generations just for clarity. Data points are shown with their 95% confidence intervals.

CES in the 19th generation of selection in LE and are shown in Figure 1.

Our results are not unique in the study of this phenomenon. Kemler (1974), while studying classical conditioning in D.melanogaster, also assayed CES in the subjects (Oregon-R, maintained at the University of Nebraska at Lincoln). Kemler likewise artifically selected for high and low CES and stated that "selection is particularly effective in the direction of reduced arousability" (p.70). We interpret this statement to indicate that Kemler had success in developing a low line but not a high line, a result similar to ours with Austin.

In summary, high and low CES lines have been established, albeit from different foundation populations, which can now be analysed genetically and used to assay the effect CES may have on other behaviors.

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References: Bennet-Clark,H.C., A.W.Ewing & A.Manning 1973, Behav.Biol. 8:763-769; Dethier,V.G., R.L.Solomon & L.H.Turner 1965, J.Comp.&Physiol.Psych. 60:303-313; ____ 1968, J.Comp.&Physiol.Psych. 66:144-150; Holliday,M., M.Vargo & J.Hirsch 1983, DIS 59:140-141; Kemler,W.M. 1974, Non-associative modificaton of behavior in Drosophila melanogaster under classical conditioning procedures, unpubl. PhD dissertation 1974, U. of Nebraska, Lincoln; McGuire,T.R. 1981, Behav.Genetics 11:331-338; Tully,T. & J.Hirsch 1982a, Behav.Genetics 12: 395-416; ____ 1982b, Anim.Behav. 30:1193-1202; ____ 1983, Behav.Neurosci. 97:146-153; Tully,T., S.Zawistowski & J.Hirsch 1982, Behav.Genetics 12:181-191; Vargo,M. & J.Hirsch 1982a, J.Comp.&Physiol.Psych. 96:452-459; ____ 1982b, DIS 58:149-150; Vargo,M., M.Holliday & J.Hirsch 1983, Behav.Res.&Methods & Instrum. 15:1-4.

Villarroel, H. and P.Zamorano. Academia Superior de Ciencias Pedagogicas, Valparaiso, Chile. Drosophila species which inhabit the National Park "La Campana".

The particular geographic configuration which presents Chile, both externally and internally (Brncic 1970), has permitted the development of a flora and fauna fundamentally endemic (Reiche 1907; Fuenzalida 1950). The Drosophilidae family constitutes a good example of this phenomenon.

The purpose of this work is to carry out a preliminary search of the Drosophila species which live in the National Park "La Campana" Valparaiso. This site is considered as one of the most interesting ecological areas in Central Chile (Rundel & Weisser 1975).

The collections were made during the period of October 1982 and March 1983. The capture was done by means of the usual trapping method with fermented banana bait.

Table 1. Total number of flies and their corresponding percentages.

Species	No. of Flies	Percentages
D.amplipennis	192	9.68
D.araucana	569	28.69
D.busckii	1	0.05
D.immigrans	428	21.58
D.pavani	27	1.36
D.repleta	66	3.33
D.subobscura	565	28.50
Total	1983	100.00

Of the 33 species described for Chile by Brncic (1957a, 1962a), 9 Drosophila species (Table 1) were collected in the National Park, which have been grouped according to Brncic (1970) in: (a) widespread species: D.busckii, D.immigrans, D.melanogaster, D.repleta & D.simulans; (b) endemic and ecologically restricted species: D.amplipennis; (c) endemic and ecologically versatile species: D.araucana and D.pavani.

We must add that on this occasion samples of D.subobscura were also collected, which correspond to a colonizing species for Chile (Brncic & Budnik 1980).

Finally we desire to point out that the place chosen for our study presents very interesting biological characteristics, such as the presence of one set of typical Drosophila species, which is found in relation to specific habitats. This event will permit us to carry out important studies on the biology of populations of these organisms.

References: Brncic,D. 1957a, Colecc.Monografia Biologicas Universidad de Chile, Santiago; ____ 1962a, Biologica 33:3-6; ____ 1970, "Essays in Evolution and Genetics" 14:401-436; Brncic,D. & M.Budnik 1980, DIS 55:20; Fuenzalida,H. 1950, Clima 1:188; Biogeografia 1:371; Reiche,K. 1907, Die Vegetation der Erde, VIII; Rundel,P. & P.Weisser 1975, Biol.Conserv. 8:35-46.

Whitmore, T. and W.-E.Kalisch. Ruhr-Universität Bochum, FR Germany. Hoechst 33258 staining of surface spread polytene chromosomes in D.hydei.

The bibenzimidole derivative Hoechst 33258 has been used extensively in the past as a DNA-specific fluorochrome in cytofluorometric investigations of metaphase chromosomes (see for example, Holmquist 1975; Latt & Wohlleb 1975; Wheeler & Altenberg 1977; Singh & Gupta 1982). Its use with polytene chromosomes has been, however, rather limited (Holmquist 1975; Lakhotia & Mishra 1980; Martin & Sedat 1982). We found that it can also be used, similar to