Conditioned discrimination in the blow fly, *Phormia regina*: Controls and bidirectional selection

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Previous behavior-genetic analyses of the blow fly, *Phormia regina*, using conditioned behavior have employed the Nelson procedure. Recent evidence suggests that measures of conditioning obtained with this procedure may be confounded with effects produced by nonassociative factors, base-level responsiveness, and a water-induced central excitatory state. Conditioned discrimination may be a way to avoid such confounds. Experiment 1 describes a taste discrimination procedure for conditioning *P. regina*, as well as the results of both a reversal and a randomized control to validate its effectiveness. Experiment 2 uses the conditioned discrimination procedure with an automatic stimulus-presentation apparatus to carry out bidirectional selective breeding for performance. Selection has produced two lines that differ significantly in conditioning performance, both from one another and from the foundation population. The results were not due to simple differences in base-level responsiveness. It is suggested that the conditioned discrimination procedure will prove useful in further behavior-genetic analyses of *P. regina*.

The behavior-genetic analysis of a species can serve to provide information about both the genome of that species and the behaviors employed for the analysis (Zawistowski & Hirsch, 1983). Behavior-genetic analyses of the blow fly, *Phormia regina*, using the proboscis extension reflex (PER) have revealed interesting and potentially important information about relationships among both associative and nonassociative processes and their gene correlates.

P. regina have been used for behavior studies reporting both associative effects, such as classical conditioning (Nelson, 1971), and nonassociative effects, like habituation (Dethier, 1952) or central excitatory state (CES; Dethier, Solomon, & Turner, 1965). The utility of *P. regina* for behavior-genetic analysis has been

documented by studies confirming the existence of gene correlates for all of the behavioral effects mentioned (Hirsch & McCauley, 1977, and McGuire & Hirsch, 1977, conditioning; McGuire, 1981, and Tully & Hirsch, 1982a, 1982b, CES; and Bryan, Zawistowski, & Hirsch, 1984, habituation). *P. regina* have also been used in combined hybridization and correlational analyses confirming a lack of independence between the gene correlates of CES and the gene correlates of conditioning (Tully, Zawistowski, & Hirsch, 1982). A common feature of all these studies has been the use of the PER, or feeding response, as the phenotypic index of behavior. *P. regina* have chemosensitive hairs on their legs, which, when stimulated by an appropriate substance (e.g., sugar for a hungry fly), elicit a PER (see Figure 1).

Unconvincing evidence of learning in Diptera once prompted Dethier (1966) to suggest that their ability to learn may have been eliminated in the interest of evolutionary parsimony. Later, however, Dethier's student Nelson (1971) reported a series of experiments showing that the PER of *P. regina* could be conditioned to taste stimuli. Although water-satiated flies normally respond at only a low rate to either water or saline (1.0 M NaCl), this response will increase dramatically over trials if saline or water presentations are paired with sucrose presentations (Nelson, 1971).

Later, Hirsch and McCauley (1977) and McGuire and Hirsch (1977) provided evidence that one could do bidirectional selective breeding for performance on this

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task. These data are important because earlier studies by Thorpe (1939) and Murphey (1967), in reporting learning in *Drosophila melanogaster*, had failed the test of independent replication and selective breeding for individual differences (IDs), as reported by Manning (1967) and Yeatman and Hirsch (1971), respectively. The selection results reported by Hirsch and McCauley and by McGuire and Hirsch indicate that IDs in conditioning performance of *P. regina* can be scored reliably and that they reflect an underlying distribution of genotypic differences correlated with the trait. The possibility remains, however, that the differences observed in these studies reflect factors other than conditioning performance. In both studies, the measure of conditioning was the total number of responses to the second of two serially presented conditioned stimuli on the final 8 of 15 trials. Although the initial average rate of responding is low within a group, there are IDs in base-level responsiveness that may affect the validity of the conditioning measure (Tully & Hirsch, 1983). Padeh, Wahlsten, and DeFries (1974) have recommended discrimination training as a way to control for nonassociative, base-level effects on performance. (Using discrimination training, Platt, Holliday, & Drudge, 1980, have since reported evidence for maze learning based on observations of individual *Drosophila*.) A comparison of the levels of response to a CS+, presented in tandem with a US, with those to a CS-, unpaired with a US, should reveal a difference that would reflect the effects of conditioning. Conditioned discrimination training offers other advantages for further analyses of *P. regina*. Tully and Hirsch showed that, in addition to sucrose-induced CES, a water-induced CES might affect measures of conditioning. That water will set up a CES is particularly troublesome for the interpretation of the Nelson (1971) study. Nelson had used serial compound CSs with a CS₁ followed immediately by a CS₂. Distilled water and 1.0 M NaCl served as the CSs. It is possible that the CS₁ sets up a CES and, if so, that responses to the CS₂ confound both conditioning and an additional CES component. Therefore, a training procedure that limits the effects of both base-level responsiveness and water-induced CES might be of great advantage in further behavior-genetic analyses of *P. regina* using conditioned behavior.

Although evidence for conditioned discrimination by various blow fly species has been reported by Frings (1941), Maes and Bijpost (1979), Nelson (1971), and Nobrega (1975), these studies lack important controls. Problems with the interpretation of the Frings study were discussed by Nelson. Nelson, while providing extensive control evidence for the excitatory conditioning described above, does not present controls for conditioned discrimination, other than to test balanced groups. Nobrega does present a sensitization control; however, his evidence is based on a single stimulus configuration with NaCl as the CS+ and water as the CS-. Maes and Bijpost present no control data, although they use a variety of salts as the CS+ (with 1 M NaCl as the CS-).

Each of the above studies presents interesting and otherwise suggestive evidence. Given the often tenuous nature of claims for learning in flies, however, we now use two important, and hitherto missing, control procedures to demonstrate that *P. regina* are indeed capable of conditioned discrimination: A reversal is employed interchanging in a later conditioning period the roles of stimuli A and B, the CS+ and CS- for the earlier conditioning period, so that B becomes the CS+ and A the CS-. We also use a randomized control. Since the differential rate of response observed in the conditioned discrimination training is believed to result from the pairing of the US with the CS+ but not with the CS-, we removed this difference in a control group by pairing both stimuli equally often with the US. In addition, we subject the conditioned discrimination performance to an analysis of reliably measured IDs by carrying out a bidirectional selection experiment, using a modified version of the procedure on an automated stimulus-presentation apparatus.

EXPERIMENT 1

Method

Subjects. The subjects were adult blow flies (*P. regina*) from the SYN stock, a free mating, wild-type culture derived from a cross of the C and DT lines listed in McGuire and Hirsch (1977, Table 1). Eggs were collected on 10-20-g pieces of pork liver placed in a cage of adults. The next day, 75-100 first instar larvae were placed on 200 g of liver in a glass battery jar (20 × 15 cm), covered with sawdust and allowed to mature at room temperature (19°-25°C) for 6-8 days. At this time, 30-50 puparia were collected and placed in a 180-ml glass specimen bottle with a foam stopper. These were then placed in a controlled environment chamber (25°C and 50% RH, LD 16/8) until eclosion (2-3 days later).

Most of the flies in a batch would eclose 0800-1200 h. Then they were chill anesthetized, sexually segregated, and placed into insect cages (30.5 cm³, American Biological Materials Co.) in the environment chamber. Flies were allowed sucrose until 1200 h on the day after eclosion and water ad lib until the time of testing. The subjects were tested at approximately 72 h of age (after eclosion). The experiments were conducted at room temperature (19°-25°C).

Procedure. On Day 1 of testing, the subjects were caught by hand, removed from the insect cages and placed into plastic pipet tips (Figure 1). The pipet tips had been trimmed, creating a tapered tube into which a fly could be gently pushed forward with a small paint brush until the fly's thorax was snugly wedged at the narrow end, where the first pair of legs and the head protruded and the rest of the body was held within the pipet tip. The tips were then attached to pencils and the flies were allowed to rest for 1 h. The subjects were then water-satiated using the following procedure: 2.5-min exposure to distilled water, 2.5-min rest, and 2.5-min exposure to distilled water. After water satiation, 20 pencils, each carrying a fly (10 males and 10 females) were mounted on a wooden board (110 × 95 × 4 cm), supported by ring stands on a table at the experimenter's eye level. This was done by pushing the free ends of the pencils into holes drilled in the face of the board. Testing began at about 1100 h. CSs contained in 100 × 13 mm test tubes were presented for about 5 sec each. The test tubes were filled with appropriate solutions and were raised by hand to contact a fly's legs, at which point they were gently shaken so that the surface tension would be broken and the legs would be immersed. The US (.5 M sucrose) was delivered at the end of a glass capillary tube to the labellum (mouthparts) of a fly about .5 sec after the CS+ (trace conditioning). On each trial, responses (PERs) to the CS or US were recorded as all or none. Response scores were based on Dethier et al.'s (1965) 7-point scale of proboscis extension, where 0 corresponds to no extension and

Figure 1. Blow fly mounted in plastic pipet tip and showing a PER. Note that the forelegs are exposed for stimulation and the remaining pairs of legs are restrained within the pipet tip.

6 is full extension with labellar lobes spread. In this study, only extensions corresponding to Positions 3-6 were scored as a response. Figure 1 presents a fly with its proboscis extended to Level 4. CS presentations were timed with the aid of a metronome set at 1 Hz. Trials (successive CS presentations) were separated by a 5-min intertrial interval (ITI) timed with a stopwatch. A 30-trial session lasted 2.5 h. At the end of the first session, the animals were removed from the pipet tips and stored separately overnight, each in a labeled, foam-stoppered, glass vial containing a wad of moistened cotton. The following day (Experimental Day 2), they were remounted, water-satiated, and conditioned with the stimuli reversed.

Stimulus schedules. In a 30-trial session, there were 15 presentations of the CS+ and 15 of the CS- (1.0 M KCl or 1.0 M NaCl). Half of the total subjects in the experimental group received KCl as the CS+ and NaCl as the CS- on Day 1 (Subgroup KNE), and half received the opposite arrangement (Subgroup NKE). These presentations were randomized, allowing no more than three consecutive presentations of one stimulus. For data analysis, a session was divided into three blocks of 10 trials, each block with five presentations of both CSs. Although each subgroup received the same sequence of CS+ and CS- trials, they differed in whether KCl or NaCl was presented. A random control subgroup was matched to each experimental subgroup (i.e., KCl was received by Control Subgroup KNC on the same trials as Experimental Subgroup KNE, and by NKC on the same trials as NKE). For the controls, however, in the first two blocks (20 trials), the US was randomly paired with each of the CS stimuli (five pairings with KCl and five with NaCl). Thus, the two stimuli provided equivalent predictive information regarding the US. On the third block, however, the control subgroups received the same CS+-US pairing sequence as their matched experimental subgroups. This was done to assess the effects of the differences in previous stimulus presentations. Twenty animals were conditioned at a time, five from each of the four subgroups KNE, KNC, NKE, NKC. The animals within a subgroup were mounted next to one another in blocks of five. Moving from left to right along the board, the subgroups were placed in the order KNE, KNC, NKE, NKC. Four such batches, a total of 80 subjects, were observed.

Results

Responses to each stimulus (CS+, CS-, and US) were summed within each block. Animals failing to respond to the US on at least 12 of the 15 presentations each day were omitted from the analysis (a total of three flies), because this might have represented a pathological con-

dition (see McGuire & Hirsch, 1977). In addition, one animal escaped and another died before completion of the 2-day sequence. Additional subjects were randomly discarded to equalize sample sizes within the cells of the statistical analysis. This left $n = 17$ subjects per subgroup, for a total $N = 68$. Tables 1 and 2 present, for the experimental and control groups, respectively, the mean number of responses made to the CS+ and CS- within each block. A mixed design for repeated measures (one within-subjects variable, blocks; one between-subjects variable, stimulus order) was used to analyze the experimental and control groups separately (Myers, 1979, p. 199). The summed responses for an individual, during a block, were expressed as a proportion (number of responses to the CS divided by 5, the total number possible during a block) and transformed using the arcsine square root, as suggested by Myers (p. 73). The analyses were then performed on difference scores calculated from the transformed data. This allows us to analyze the change in differential response levels, over the blocks of trials.

The experimental group shows a block effect [$F(5,160) = 6.75$, $p < .001$], indicating that conditioned discrimination had occurred, inasmuch as no such effect appeared in the random control group [$F(5,160) = 1.56$, n.s.]. Since the analyses reveal no stimulus order (subgroup) differences within a treatment [experimental, $F(1,32) = .02$, n.s.; control, $F(1,32) = .56$, n.s.], the data within treatments were pooled and the combined experimental and combined control data compared in Figure 2. This combined analysis shows both a block effect [$F(5,330) = 5.52$, $p < .001$] and a block $\times$ treatment interaction [$F(5,330) = 2.45$, $p < .05$], indicating both that a change had occurred over trials and that it differed between treatments. Within each day, the experimental groups show a significant block effect, rising monotonically [$F(2,64) = 5.73$, $p < .005$; $F(2,64) = 15.32$, $p < .001$, for Days 1 and 2, respectively], further confirming the interpretation that conditioning had occurred each day. The random control groups show no block effect on Day 1 [$F(2,64) = 1.01$, n.s.], but there is a significant block effect on Day 2 [$F(2,64) = 3.69$, $p < .05$],

Table 1
Mean Number of Responses with Standard Error of the Mean (SEM) to the CS+ and CS- for the Combined Experimental Groups ($n = 34$)

Block	CS+		CS-	
	Mean	SEM	Mean	SEM
1	2.03	.29	2.41	.31
2	2.64	.31	2.71	.25
3	3.38	.28	2.82	.31
4	2.65	.28	3.47	.27
5	3.62	.31	3.53	.29
6	3.88	.24	3.35	.31

Note—Blocks 1-3 are from Day 1 (test) and Blocks 4-6 are from Day 2 (reversal).

Table 2
Mean Number of Responses with SEM to the CS+ and CS-
for the Combined Control Groups (n = 34)

Block	CS+		CS-	
	Mean	SEM	Mean	SEM
1	1.97	.27	1.79	.29
2	2.15	.31	2.29	.27
3	2.41	.27	2.53	.31
4	2.23	.30	2.56	.31
5	3.29	.34	2.94	.34
6	3.12	.33	3.12	.33

Note—Blocks 1-3 are from (test) Day 1 (pairing of US with CSs is random during Blocks 1 and 2, but nonrandom during Block 3) and Blocks 4-6 are from (reversal) Day 2 (pairing is random during Blocks 4 and 5, but nonrandom during Block 6).

which Figure 4 shows to be a curvilinear change, not the monotonic increase expected if conditioning were occurring. In summary, the consistent pairing for the experimental group produces a differential response in the expected direction.

Discussion

Given consistent pairing of a CS+ with a US, *P. regina* will increase differentially their probability of a response to that CS+ when compared with their response to an unpaired CS-. Furthermore, if these CSs are reversed in their roles, *P. regina* will adjust its behavior in response to the new stimulus contingencies. These are valuable observations, because, as stated previously, the use of a discrimination task can serve to limit the effects of nonassociative responsiveness when assessing IDs in conditionability. This procedure should lend itself to more refined genetic analyses because it allows a more accurate categorization of IDs. Experiment 2 uses a modified version of the conditioned discrimination procedure to screen individuals for a bidirectional selective breeding experiment.

Figure 2. Mean difference scores (untransformed) and SEM for blocks of 10 trials for the combined experimental and random control groups (n = 34, each). Blocks 1-3, Day 1 (test); Blocks 4-6, Day 2 (reversal).

EXPERIMENT 2

Method

Médioni and Vaysse (1975) used a vertical kymograph to stimulate automatically an individual *D. melanogaster*. A fly is held stationary by attaching it to the end of an insect needle. Pads soaked with appropriate stimulus solutions are placed on the surface of a kymograph drum. The fly is then placed horizontally on an axis tangential to the drum surface such that, as the drum rotates, the fly's legs can contact the stimulus pads. This method has been modified by Vargo, Holliday, and Hirsch (1983) to permit stimulating several individuals together, by using strips of filter paper, rather than pads, to present stimuli. These strips are wrapped obliquely around the drum in a manner similar to the stripes on a barber's pole. When moist, they will adhere to the drum's surface. As the vertical drum rotates, flies mounted and placed on horizontal rods at parallel tangents are stimulated in a staggered fashion, allowing one to observe both the stimulation and response of each fly in sequence. The strips are kept moist by continually being saturated with stimulus solution from reservoirs located at the top of the drum. Capillary tubes carry solutions from the reservoirs to the top of the strips. Gravity then causes the solutions to flow down the strips to a catch pan below the drum. A rubber band placed between the adjacent CS+ and US strips prevents the mixing of stimuli. By appropriate spacing of strips and manipulation of drum rotation speed, control can be exerted over intertrial and interstimulus intervals. A windfall resulting from the use of the drum is that, if *P. regina* are allowed to walk along it (as if on a conveyor belt), the activity should help to discharge CES (see Nelson, 1971, Experiment 7).

A kymograph (Phipps & Bard, Inc.) was set up as generally described in Vargo et al. (1983). Two drums were bolted together from the inside to form a single unit 31 cm tall. Stimulus strips were then spaced and placed on the drum along with a rubber band between the strips corresponding to the CS+ and US. The drum was then spray-painted with a flat white paint. When the paint dried, the paper strips were removed. The resulting unpainted areas could then serve as a template for the application of stimulus strips for testing. The edges of the rubber band were further sealed with Silastic (Dow Corning) to prevent seepage of sucrose from the US strip to the CS+. The effectiveness of this partition was confirmed by running a dye through the US strip and observing no contamination of the CS+. No dye was used during actual testing. The drum was set to rotate about 10 cm/min, or one revolution each 5 min. CS strips (Whatman No. 3 filter paper) were cut 7 mm wide; the US strip was half that width. Since a fly mounted in a pipet tip can move its forelegs forward to meet a strip as it approaches or drag them to retain contact as it passes, CS stimulations generally ranged 2.5-6 sec and US stimulations, 1-3 sec. The rubber partition between the CS+ strip and US was 2 mm wide, providing for a 1-3-sec trace. The CS- strip was placed halfway around the drum, making an interstimulus interval of 2.5 min between the US and the CS- and one of 2.5 min between the CS- and the CS+ of the succeeding trials. Thus, a single drum rotation corresponded to one trial with a 5-min ITI. Strips were applied to the drum by being moistened with the appropriate stimulus solution and being placed on the template at the desired location. The reservoir was then set above the strip on the top of the drum. A glass capillary tube was heated to be bent and drawn to a narrow tip. Adjustment of the tip size controlled the rate of flow at about .5 ml/min. The supply of solution in a reservoir was replenished when necessary with a wash bottle. As in Experiment 1, the CSs used were 1.0 M NaCl and 1.0 M KCl with a .5-M sucrose US.

The foundation population (FPOP) consisted of 120 individuals from SYN. They had been sexed within 5 to 6 h of eclosion and placed in separate cages. Sucrose was allowed ad lib until 1200 h of the following day, at which time it was removed and the flies were food deprived for about 48 h, until the time of testing. Water was provided ad lib during this entire period. On the day of testing, the animals were mounted 60-90 min prior to testing, which occurred between 1000 and 1600 h. They were allowed to rest during this period under normal lighting, at room temperature. Using the drum setup described, 15 flies could be conditioned in a 75-min session. Usually, two such sessions were conducted, yielding a total of 30 individuals each day. Before the first session began, the flies to be tested in the second session were mounted

and allowed to rest while the first session was being conducted. Just prior to the start of a session, the flies to be tested were given a brief (2.5-min) exposure to water to ensure satiation. Very few animals drank at this time. The flies were then placed on pencils tangential to the drum. Their position was adjusted so that their forelegs would contact the surface of the drum and the stimulus strips. If an animal lifted its legs as a stimulus strip approached, that presentation was scored as a "non-participation" (see Vargo et al., 1983). Nonparticipations were rare. All subjects in FPOP and in the even generations of selection were conditioned using KCl as the CS+ and NaCl as the CS-; for the subjects in the odd generations of selection the stimuli were reversed, with KCl serving as the CS- and NaCl as the CS+.

The selection criteria employed were our modification of McGuire's (1978, pp. 46-51) elaboration of the classificatory system used by Nelson, who had counted responses to the second of the compound stimuli (the CS₂) on the last 8 of 15 trials, and classified 6 to 8, 3 to 5, and 0 to 2 responses as representing good, fair, and poor conditioners, respectively. Our study also considered the number of responses to the CS-. Individuals with a CS+ score of 6 or greater, a CS- score of 3 or less, and a difference score of 4 or greater were classified as high performers. This ensures that animals used to breed the high discrimination line (HD) are responding at a fairly high rate at the end of a session, but only to a particular stimulus, the CS+. Low discriminators (LD) are those having a difference score of -2 to 2 with CS+ and CS- scores of 1 to 3 (Zawistowski, 1983, pp. 49-50). Animals responding zero times to both stimuli were not used, because it was unclear whether they were able to sense the CSs. No animals were considered in the analyses or for selection if they failed to respond at least 12 times to the US or had three or more nonparticipations to any of the stimuli during the last eight trials. The animals in FPOP meeting the criteria for high or low discrimination were saved and used to breed HD and LD, respectively. These lines were maintained in reproductive isolation and tested separately over subsequent generations. Animals within each line meeting their respective performance criteria have been used as parents to produce the next generation. Enough flies were tested from each line every generation to obtain six to eight individuals of each sex for breeding. An unselected control line (CD) was kept and tested periodically. It was maintained by breeding from it each generation seven pairs of randomly chosen flies. It has been used as a control to assess effects of environmental changes and any possible genetic "bottle-neck" effects resulting from the use of only six to eight pairs for breeding each generation.

Results

Figure 3 depicts the performance curves for FPOP. It can be seen that response to the CS+ starts near 0% and climbs steadily until 40% of the sample ($n=108$ after discards) is responding to it on the 15th trial. Response to the CS-, on the other hand, remains at approximately 20% throughout. The level of response to the CS- may be a result of the CES's being incompletely discharged by the walk on the drum. However, by the last eight trials (those used for the selection criteria), the animals were responding significantly more often to the CS+ (paired $t=5.32$, $p < .001$). It should be noted that the reliabilities (Spearman-Brown formulation; Hirsch & Tryon, 1956) for the CS+ and CS- over these last eight trials are .93 and .88, respectively. Fifteen individuals (8 females and 7 males) were chosen to found HD and 14 (7 females and 7 males) were chosen for LD. Selection then continued for 11 generations. Shortly after testing the individuals of the sixth generation, difficulties were experienced in the laboratory related to the maintenance of these and other stocks. During this time, several stocks died (including CD) and others were almost lost. Dif-

Figure 3. Performance curves for FPOP, with response to the CS+ (KCl) rising and that to the CS- (NaCl) is relatively stable.

ficulties included a drop in egg production, failure of the eggs to hatch, death of larvae at intermediate instars, prolonged developmental periods, and failure to eclose once puparia had been formed. It is known that insecticides were sprayed in the building at least once during this period. The difference scores for HD₇ drop and those for LD₇ and CD₇ both rise. It was not possible to test Generation 8 of any line because there were too few progeny produced by Generation 7. Selection was then resumed with HD₉ and LD₁₀ and continued for each line until Generation 11. At this time their performance was compared with that of SYN (see Table 3), from which they were derived, and which had been maintained by mass mating.

Figure 4 shows that over the generations of selection in both HD and LD there is an initial increase in mean difference scores. Subsequently, LD decreases while HD continues to increase. A regression analysis reveals a significant increase in the mean difference score over generations for HD ($b=.19$, $F=46.39$, $p < .001$) and a significant decrease for LD ($b=-.52$, $F=4.62$, $p < .032$). At the 11th selected generation, the HD and LD mean difference scores differed from one another and from that of SYN₂₇ (ANOVA, omnibus $F=34.71$; LSD test, $p < .05$). However, the lines do not differ in mean response to the CS- (omnibus $F=1.004$, LSD test, $p > .05$). LD and SYN do not differ in response to the CS+, but HD shows a significantly greater mean than either (omnibus $F=18.761$, LSD test, $p < .05$). This is the result of a significant rise over generations of the CS+ mean in HD ($b=.11$, $F=10.96$, $p < .001$) and a decrease in the mean response to the CS- ($b=-.79$, $F=15.19$, $p < .001$). At the same time, LD became less responsive to both the CS+ and the CS- (CS+, $b=-.15$, $F=19.78$, $p < .001$; CS-, $b=-.95$, $F=14.80$, $p < .001$). Figure 5 depicts the performance curves for HD₁₁ and LD₁₁. It shows that responding to the respective CSs starts near the same level for both HD and LD.

Table 3
Means and SEMs Over the Last 8 of 15 Trials for FPOP,
SYN₂₇, HD₁₁, and LD₁₁ (DIFF = CS+ - CS-)

Line	CS+		CS-		DIFF	
	Mean	SEM	Mean	SEM	Mean	SEM
FPOP	2.79	.30	1.54	.22	1.25	.23
SYN ₂₇	2.46	.52	.63	.24	1.83	.39
HD ₁₁	4.72	.37	.62	.19	4.10	.39
LD ₁₁	1.70	.33	1.03	.28	.67	.14

HD shows a large increase in level of responding to the CS+, but LD does not. Although FPOP showed a maximum near 40% response to the CS+, HD reached 70%. The comparable levels of response to the CS- by HD and LD indicate that selection in HD was not for simple responsiveness.

Discussion

As the evidence shows, bidirectional selection for conditioned discrimination has succeeded. Eleven generations of selection produced two performance lines that differed significantly from one another and from the unselected foundation population from which they were derived. More importantly, these lines do not differ due to increases or decreases in simple overall responsiveness. When compared with LD and SYN, HD shows a greater mean response to the CS+ but not to the CS-. Since KCl and NaCl were reversed in their positions as either the CS+ or CS- on alternate generations, the results are not due to selection for stimulus bias. The CS+ and CS- differed only in their contiguity to the US. In addition, the performance curves for HD₁₁ and LD₁₁ (Figure 5) show that their responsiveness on the first trial was about the same and that, over the course of training, the responding to the CS+ showed a large increase by HD₁₁, but not by LD₁₁. Moreover, response to the CS- by both lines remains at the same consistently low level. It is important to realize that in fact we may not have selected for "conditionability." Although selection was not for simple responsiveness, the possibility exists that selection may have affected one of the support systems involved, such as discrimination ability. HD and LD may have the same conditioning potential, but if the individuals of HD are better discriminators, they will perform better in the procedure used.

GENERAL DISCUSSION

In the Diptera, studies of learning or conditioning can be combined with genetic analyses. The ability reliably to screen individuals using a valid procedure should greatly increase the efficiency of the genetic manipulations used. Based on the previous work of others, and using our own important controls, we established a procedure valid for the conditioned discrimination training of *P. regina*. Our demonstration of conditioning in Experiment 1 reveals a modest, though significant, effect in an

unselected population, from which one might conclude that "blow flies" are therefore capable of only "modest" amounts of conditioning. Such a conclusion, however, would represent an inappropriate exercise in typological thinking. One should recognize such data as the average performance of a population of individuals, each with its own performance characteristics. One should not equate species capacity with the mean performance of a particular sample or population. In Experiment 2, we demonstrate that, when one can detect and breed selectively on reliably measured, valid performance criteria, the resulting populations may differ quite substantially from the original foundation stock. In HD, what was once a modest effect has now become quite robust. On the other hand, in LD, there is only a small conditioning effect. What becomes

Figure 4. Selection curves: Mean difference scores on the last eight trials, showing the change in mean difference score [with 95% confidence interval (CI)] for HD, CD, and LD. For FPOP and even generations, CS+ was KCl and CS- was NaCl, with stimuli reversed on odd generations. After Generation 7, when CD was lost, SYN₂₇ was tested. (Some CIs have been displaced for clarity.)

Figure 5. Performance curves for HD₁₁ and LD₁₁. Although in FPOP response to the CS+ reached about 40%, in HD it approached 70%. At the same time, the CS- response shows an equivalently low level in both HD and LD.

important is not necessarily the ability to demonstrate a large average effect in the initial population, but rather to develop a procedure that allows one to identify various types of performers. Analyses are currently underway to increase our resolution of IDs in performance. Continued investigation may lead to new insights into the phenomenon of conditioning and its gene correlates.

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