

Some approaches to the question of *Drosophila* laterality

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(Received 30 January 1978)

SUMMARY

The question of *Drosophila* laterality has been investigated in two species and in several strains by attempting to modify degrees of laterality (wing folding and directional choices in mazes) via selection and by ascertaining individual courtship behaviour preferences (circling, foreleg tapping, and wing extending). The results of courtship studies in *D. paulistorum* strains and in *D. melanogaster* indicate that, although individual members of a strain may be entirely right or left dominant, the majority of individuals are ambidextrous. As a group, there was no preference for right or left behaviours, and laterality was not a factor in success of courtship. The absence of lateral preference in courtship is consistent with the failure to demonstrate any genetic component for *D. melanogaster* left and right wing folding and maze behaviours.

1. INTRODUCTION

Asymmetrical lateral biases appear to exist both as morphological traits and as behavioural traits. A typical example of the morphological expression of asymmetry is the pair of dimorphic claws of the lobster, *Homarus americanus*, a cutter and a crusher (Lang *et al.* 1977). Behavioural expression of asymmetry (pawedness or handedness) has been observed and investigated in chimpanzees, parrots, rats, monkeys, humans, and cats. All except cats have shown a preference for a specific limb (Cole, 1955). Human handedness may also be a factor of asymmetrical brain development (see, Dimond & Beaumont, 1974; Harnard *et al.* 1977; and Manowitz, 1977, for reviews).

Although laterality *per se* has not previously been investigated in any *Drosophila* strain, Purnell & Thompson (1973) did study genetic components of lateral asymmetry in *D. melanogaster*. Beginning with a wild-type strain, two lines (left over right and right over left wing folding) were selected for 15 generations. A small asymmetrical bias in wing folding direction was initially achieved, but was lost after the first few generations. Artificial selection to modify bristle and hair number asymmetries has also been attempted with some success in *D. melanogaster* (Beardmore, 1965; Reeve, 1960; and references in Purnell & Thompson, 1973).

In addition to wing folding direction, *Drosophila* may be scored for directional choices in mazes and for several laterally classifiable behaviours that are sex specific. *D. melanogaster* and *D. paulistorum* employ three male behavioural traits in courtship that can be unambiguously classified as right or left behaviours: circling, tapping, and wing extension. Part of this study was designed to search for any lateral preferences in *D. melanogaster* and *D. paulistorum* when executing the three courtship behaviours. For additional analyses the subjects were further divided into two groups: successful courtship, defined by copulation within thirty minutes of introduction to a mature female, and unsuccessful courtship. The second part of the study involved the use of selection lines to investigate the genetic component, if any, of two related behaviours: wing folding, which may be correlated with wing extension, and maze direction choice, which reflects an ability of the organism to 'recognize' left and right.

A thorough review of the literature revealed no evidence upon which to base an hypothesis relative to courtship behaviour asymmetry or maze direction choice; this study may, therefore, be considered an initial one. It was expected that there would be no significant differences in lateral preferences in courtship behaviours of circling, tapping, and wing extension in *D. paulistorum* and in *D. melanogaster* when divided by successful and unsuccessful courtship, and that, consequently, one would predict no significant heritability of wing folding direction or maze direction choice behaviours in *D. melanogaster* (see, Hay & Crossley, 1977, for an overview).

1. MATERIALS AND METHODS

(i) *Components of courtship behaviour*

The *D. paulistorum* strains represented the Mesitas, Colombia, Andean Brazilian semispecies and the Santa Marta, Colombia, Transitional semispecies. They produce sterile male hybrids when crossed (with difficulty). The *D. melanogaster* strain is known as '+B', a wild-type isofemale strain collected from Ceres, New York, in 1968 (see, Ehrman, 1971, 1973, 1978, for details of its maintenance).

Using the single couple direct observation chambers and the technique of Koref-Santibanez (1972*a, b*), we scored for those aspects of male courtship which were clearly and cleanly classifiable as right or left gestures, i.e.

(1) Circling – the male circles the female in what seems to be an effort to limit her movements;

(2) Tapping – the male extends one foreleg and touches the nearest part of the female's body; and

(3) Wing extension – the male extends a single wing sideways (at about 90°) and then returns it to the closed position (see, Ehrman & Strickberger, 1960, for pictures of the above).

Flies were aged for 2–3 days (*D. melanogaster*) or for 5–6 days (*D. paulistorum*) while isolated from members of the alternate sex. They were then introduced into the steep, small chambers without anaesthetization. Each couple was observed under low magnification for 30 min or until copulation occurred. We also tried to

score equivalent behaviours in a strain of *D. pseudoobscura* but the rate of performance was low or nil.

(ii) *Artificial selection*

Wing folding and maze behaviour selection lines were initiated from a base population derived by crossing progeny from several wild *D. melanogaster* lines caught during the spring of 1976 in central Oklahoma. They were maintained on an agar, cornmeal, molasses medium at 25 ± 1 °C.

Two selection lines for wing folding preference were maintained: one for left wing folded on top of the right wing (L/R) and one for the right wing on top (R/L). In each line the parents were selected from samples of 50 virgin flies of each sex. After enough virgins had been collected, they were lightly etherized, scored, and the two phenotypic groups (L/R and R/L) were placed in separate food tubes. About 24 h later they were lightly re-etherized and scored again. Five pairs of parents ($\frac{1}{10}$ of all scored flies) were taken at random from flies that had shown the selected phenotype in both assays. The overall proportion that showed a consistent preference was plotted as the phenotype of the generation and compared to the proportion (25 %) expected on the basis of chance alone.

Heritability for wing folding preference was also measured. A large number of 1- to 2-day-old virgin flies were lightly etherized and scored on each of four consecutive days. When similar groups were pooled (based upon frequency of direction, but ignoring the order of the L/R and R/L phenotypes in the four assays), this gives rise to five possible phenotypes: L/R all four times, L/R three times and R/L once, and so forth. Single pairs were assortatively mated within these groups, and their offspring were similarly etherized and scored on four consecutive days. Heritability was estimated from the regression of offspring on midparent (Falconer, 1960; Ehrman & Parsons, 1976).

Selection was also carried out for left and for right choice in a maze, avoiding, we believe, many of the pitfalls that have plagued similar experiments in the past (see discussion in Murphey, 1969, 1973). The maze was constructed of Y-shaped polypropylene tubing connectors arranged so that each individual must make three direction choices in order to reach food tubes. One-way movement was insured with small funnels, and the maze was disassembled and cleaned before each run. Virgin females and males were run through the maze separately. At the end of each run, flies were collected from the food vials attached to the end of the last tube and counted. Five pairs of flies were taken at random from the appropriate samples (high left- or high right-preference) as parents for the next generation.

The proportion making a consistent left-preference or right-preference movement through the maze was calculated, as were the proportions making all other combinations of left and right. These proportions then provided the data for calculating an average 'direction preference phenotype' for the generation. Specifically, a left choice was given the value -1 and right choice the value of $+1$. Thus, the percentage of flies in the tube resulting from three left choices was weighted by -3 , that from the tubes resulting from two left and one right choice

was weighted by -1 , and so forth. Consequently, the sign and magnitude indicates the directional preference and a value of zero for the experimental run indicates no overall directional preference.

3. RESULTS

(i) *Laterality in courtship behaviour*

Fifty-six pairs of each strain of *D. paulistorum* and forty pairs of *D. melanogaster* were placed in observation chambers, one pair at a time, and each male's circling, tapping, and wing extending courtship behaviours were observed and tallied. Table 1 summarizes the data describing mean courtship times and numbers of successfully and unsuccessfully courting males. A χ^2 test shows no significant between-strain differences in the distribution of successfully and unsuccessfully courting males of the Santa Marta and Mesitas strains of *D. paulistorum* ($P > 0.1$). There were no unsuccessfully courting *D. melanogaster* males.

Table 1. Means and standard deviations of courtship times of successful and unsuccessful courtships *D. paulistorum* and *D. melanogaster* (N is given in parentheses)

Strain	Courtship time (min)	
	Successful	Unsuccessful*
<i>D. paulistorum</i> (Santa Marta)	10.56 \pm 9.08 (39)	30.00 (17)
<i>D. paulistorum</i> (Mesitas)	10.00 \pm 6.93 (31)	30.00 (25)
<i>D. melanogaster</i> (+B)	6.70 \pm 6.43 (40)	—

* A value of 30 indicates that no successful courtship was observed during the 30-minute observation period.

In Table 2, the frequencies of right and left behaviours are compared for each set of courting couples. The means represent the average number of a particular behaviour, right circling for example, counted for a courting male. Thus, for successfully courting Santa Marta couples, an average male made 2.00 right circling approaches and 2.18 left circling approaches to the courted female. The t -values are shown for each right-left comparison; none are significant at the 0.05 level. Thus, although between 20 and 34% of the subjects in each condition displayed only right or only left behaviours, as a group no preference was shown.

One interesting observation, however, is the extreme proportion of wing extending behaviour compared to other courtship behaviours exhibited by *D. melanogaster* (approx. 30:1). The proportion of wing extending behaviour in *D. paulistorum* is much lower (0.5:1 in Mesitas; 2:1 in Santa Marta), while tapping behaviour is more common.

(ii) *Differences in courtship success*

In Table 3, a comparison is made between the frequency of right and left behaviours in successful versus unsuccessful courting males. The laterality values upon which these t -ratios are calculated are shown in Table 2. Thus, e.g., a successfully courting Santa Marta male made an average of 2.00 right circling approaches, whereas an unsuccessful male made 7.71 right circling approaches ($t = -2.10$).

Table 3, therefore, shows that within the Santa Marta strain, a significant difference exists between groups of males in all behaviours, except left circling behaviour. The 'total behaviour', i.e. the sum of the three targeted behaviours, of successful and unsuccessful males is also significantly different ($P < 0.01$). This indicates continued courting activity by the unsuccessful Santa Marta males. It is

Table 2. Means, standard deviations, and *t*-ratios of differences between means of left and right circling, tapping, and wing extending behaviours in successful and unsuccessful courtships

Strain and behaviour	<i>N</i>	Right behaviour	Left behaviour	<i>t</i> *
Santa Marta, successful				
Circling	39	2.00 ± 6.29	2.18 ± 5.26	-0.14
Tapping	39	3.44 ± 8.13	3.13 ± 5.09	0.29
Wing extending	39	2.08 ± 3.21	2.95 ± 4.29	-1.02
Santa Marta, unsuccessful				
Circling	17	7.71 ± 10.40	8.71 ± 13.56	-0.32
Tapping	17	13.76 ± 14.30	10.53 ± 8.90	0.79
Wing extending	17	8.76 ± 10.34	8.18 ± 8.88	0.20
Mesitas, successful				
Circling	31	1.55 ± 4.36	1.84 ± 5.33	-0.24
Tapping	31	2.39 ± 3.32	1.58 ± 2.00	1.16
Wing extending	31	1.87 ± 3.37	2.19 ± 6.44	-0.25
Mesitas, unsuccessful				
Circling	25	0.56 ± 0.92	0.56 ± 1.16	0.0
Tapping	25	4.32 ± 4.39	3.64 ± 4.97	0.49
Wing extending	25	1.24 ± 2.22	1.28 ± 2.62	-0.06
+ B, successful				
Circling	40	0.20 ± 0.46	0.30 ± 0.82	-0.67
Tapping	40	0.35 ± 0.74	0.20 ± 0.61	1.00
Wing extending	40	10.45 ± 12.33	11.32 ± 17.54	-0.26

* $P > 0.05$ for all *t*.

Table 3. Values of *t* for differences between means of circling, tapping, wing extending and of total behaviours in successful vs. unsuccessful courtships of *D. paulistorum* strains.

(Means and standard deviations upon which these *t*-ratios are computed are found in Table 2)

Behaviour	Santa Marta strain		Mesitas strain	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
R. circling	-2.10	< 0.05	1.23	> 0.05
L circling	-1.92	> 0.05	1.30	> 0.05
R tapping	-4.99	< 0.001	-1.68	> 0.05
L tapping	-3.25	< 0.01	-1.95	> 0.05
R wing extension	-2.61	< 0.01	0.67	> 0.05
L wing extension	-2.31	< 0.05	0.72	> 0.05
Total right	-3.10	< 0.01	-0.13	> 0.05
Total left	-2.81	< 0.01	0.05	> 0.05

very interesting to note, however, that similar significant differences do not exist for the Mesitas strain males, who apparently do not continue to court after initial failures.

(iii) *Selection line responses*

The purpose of the selection lines was to determine whether a genetic component of laterality could be detected by selecting for an increased expression of a particular laterality. Results are shown in Fig. 1. Neither set of selection lines produced clear-cut responses to selection. Indeed, the only trend one can pick out is in the selection for an increased left over right wing folding bias. In this line, to our surprise, it was the selected left over right phenotype that tended to decrease in later generations. Though little weight can be put on such a trend, it clearly supports the conclusion that the genetic basis, if any, of wing folding and maze choice laterality is slight.

This lack of positive selection response is confirmed for wing folding by the heritability assay made by an offspring on parent regression. Although 6% of the progeny were right or left dominant throughout the full sequence of four wing folding assays, the heritability was not significantly different from zero ($h^2 = -0.03$).

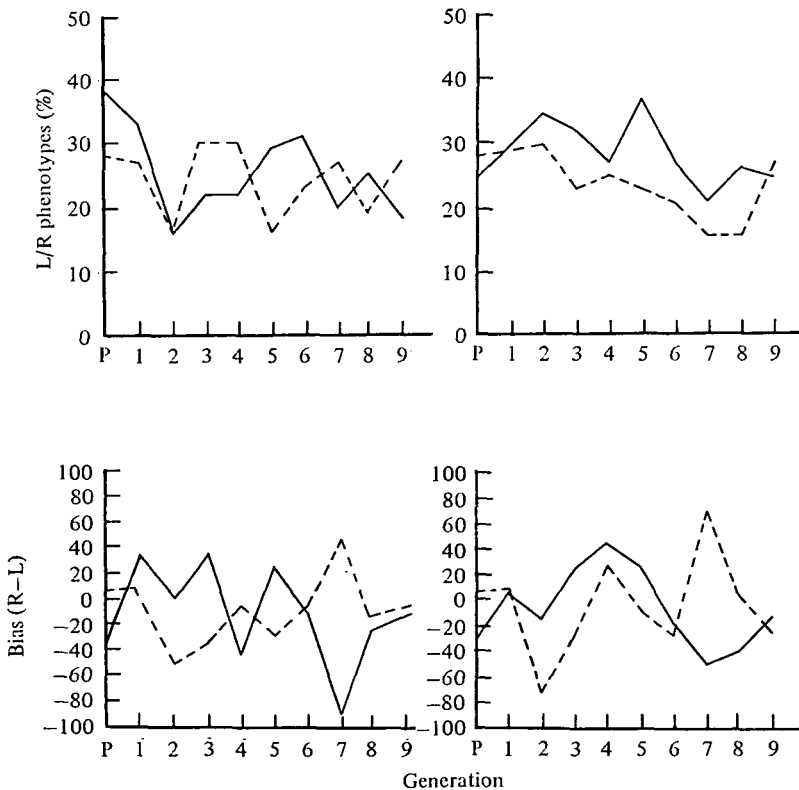


Fig. 1. Responses to selection for increased left or right preference in *D. melanogaster*. Top left, right over left wing folding; top right, left over right wing folding; bottom left, left maze choice preference; bottom right, right maze choice preference. (Solid line = female responses; dashed line = male responses.)

4. DISCUSSION

In this study we have approached the question of laterality in *Drosophila* behaviour by attempting to identify preferences in individual members of a strain and by attempting to modify the degree of laterality by artificial selection. These complementary approaches are tied together by the belief that strong individual laterality (if it exists) will have a significant genetic component, and that if the hypothetic genetic component is polymorphic (as it must be, in order to account for polymorphic phenotypes), selection should be effective in modifying the degree of laterality.

The results of courtship studies in *D. paulistorum* strains and in *D. melanogaster* indicate that although individual members of a strain may be entirely right or left dominant, the majority of individuals (more than 66 %) are ambidextrous as to courtship behaviours of circling, tapping, and wing extending. The results also indicate that, as a group, the subjects had no preference for right or left behaviour, and that laterality was not a factor in success of courtship. The absence of lateral preference in courtship is consistent with the failure to demonstrate any genetic component for *D. melanogaster* left and right wing folding and maze behaviour.

As pointed out briefly in the results section, one interesting sidelight of this study is the difference in numbers of behaviours found when comparing successfully and unsuccessfully courting males of the two strains of *D. paulistorum*. Unsuccessful Mesitas subjects apparently give up courting completely. This leads one to ask whether there is perhaps some cue(s) early in the courtship pattern that signals that a Mesitas female will not ever be available; are Mesitas males 'lazy' or are Santa Marta males 'overeager'? A second interesting observation is the extreme proportion of wing extending behaviour to other courtship behaviours exhibited by *D. melanogaster* (approx. 30:1) compared to the proportion of wing extending behaviour of *D. paulistorum*. Is the rapidity and universal success of *D. melanogaster* male courtship in some way related to the high proportion of wing extending behaviour?

In conclusion, there are clearly three questions that one can ask about laterality of a behavioural trait: (a) is there any laterality preference or lateral bias in the population; (b) do individuals show any significant lateral preferences; and (c) can one select a population to increase an overall tendency to left or right lateral preference? The results of this study would answer all of these questions 'No'. No population bias was observed for any trait, and though some individuals show predominantly left or right behaviours, selection to increase that preference was not successful. Thus, the genetic component of laterality for behavioural traits, if it exists, must be slight.

We are grateful for the laboratory assistance given to L.E. by Miss Anne Miller, Princeton University, during the summer of 1977, and for support provided to L.E. by a State University Awards Program, Grants-in-Aid. We are also grateful for support provided to J.N.T. and B.N.H. by a BioMedical Sciences Support Grant from the University of Oklahoma.

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