Behavior Genetics, Vol. 5, No. 1, 1975

Phototactic Responses Along a Gradient of Light Intensities for the Sibling Species *Drosophila melanogaster* and *Drosophila simulans*

P. A. Parsons[1]

Received 14 Jan. 1974—Final 8 Apr. 1974

The phototactic responses of four recently collected isofemale strains of Drosophila melanogaster *and* Drosophila simulans *were measured in a light gradient from 590 to 10 lux. High light intensities were preferred by most flies, but a small proportion of flies preferred the lowest light intensity. Based on the strains tested,* D. simulans *showed greater phototaxis than* D. melanogaster, *and within each species variability was found. The niche breadth of* D. melanogaster *appears likely to be greater than that of* D. simulans *for phototaxis in the light gradient. These results are in general qualitative agreement with earlier results published on dispersal activities from the same populations.*

KEY WORDS: phototaxis; *Drosophila melanogaster*; *Drosophila simulans*; genetics.

INTRODUCTION

McDonald and Parsons (1973) described the dispersal activities of the two sibling species *Drosophila melanogaster* and *Drosophila simulans*, and found that the dispersal of *D. melanogaster* exceeded that of *D. simulans*, especially toward a light source. No heterogeneity was found among strains of *D. simulans* without the light source, but heterogeneity occurred with it. The conclusion therefore is that the expression of genetic differences for dispersal among strains is light dependent. For *D. melanogaster*, heterogeneity was found with and without the light source, although dis-

[1] Department of Genetics and Human Variation, La Trobe University, Bundoora, Victoria, Australia.

persal increased when the light source was present. As for mating behavior (Grossfield, 1971, 1972), dispersal activity of *D. simulans* is more dependent on the presence of light than that of *D. melanogaster.*

The method used by McDonald and Parsons (1973) was to take isofemale strains of the two species from the suburbs of Melbourne and measure dispersal from one vial to another 23 cm away connected by a glass tube of internal diameter 0.7 cm. The testing period was 1 hr. In this paper, a further report on isofemale strains from Melbourne for the two species is presented. An apparatus was used in which flies have the possibility of selecting different light intensities when given the choice. In fact, three main classes of design have been used to study phototaxis: (1) where the measurement of phototaxis is a function of movement toward a directed light source, (2) where the distribution of flies in a light field is used as the measure, and (3) where the distribution of flies after they have selected one of two alternatives at a choice point is used as the measure (Rockwell and Seiger, 1973*a*). It is therefore not surprising that Hadler (1964) considered that one of the major difficulties in comparing phototaxis from different laboratories results from differences in experimental technique (see also Parsons, 1973*a*). The experiments of McDonald and Parsons (1973) were based on design class 1, and the experiments now to be described fall into design class 2.

METHOD

Four isofemale strains collected in the suburbs of Melbourne were used for each species. They were collected two generations before testing commenced, and results within strains were consistent throughout the test period. Flies were tested at 25°C and were 3–4 days old at the time of test. Sexes were tested separately using 200 flies per trial. Eight replicates of each sex for each strain were carried out, four in the morning and four in the afternoon. The testing apparatus consisted of a long perspex box, 180 cm long, 10 cm wide, and 10 cm deep. The box was marked into seven equal sections and had an inlet hole between the third and the fourth section. The roof of the box was of clear perspex, and 33 cm above the box were three fluorescent light tubes with dimmers on two of them to create a light gradient. The light intensities according to sections were

section	1	2	3	4	5	6	7
intensity (lux)	590	550	400	170	60	20	10

Flies were admitted through the inlet tube by gentle tapping and then left for 4 hr to select a light intensity. There was no temperature gradient in the box. For scoring purposes, a watchglass of food with a drop of yeast suspension was placed on the base of the box in the center of each of the

seven sections, so giving flies one of seven positions to select. The number of flies on each watchglass was scored after 4 hr.

Flies were cultured in ½-pint milk bottles with plenty of yeast. Good repeatability between trials was found, indicating the likelihood that nutritional variations between trials were not relevant.

RESULTS

Table I gives means for each strain for each position, and Table II gives an overall analysis of variance incorporating components of interest. The method of carrying out the analysis is explained at the foot of Table II. The angular transformation of the proportions at each position was carried out before analysis. By the design, there would be no overall differences expected between strains, sexes, and species, because in each trial a total of 200 flies was used, and indeed a detailed analysis of variance confirmed the lack of significance of these components. (This of course means that, with few exceptions, all flies ended up in the food cups.) Equally, the morning–afternoon comparison is omitted, being so small that it is incorporated into the error.

Table I. Mean Percentages for Four Strains Each of *Drosophila melanogaster* and *Drosophila simulans* at Each of the Seven Positions as Described in the Text

		Position						
Strain		1	2	3	4	5	6	7
D. melanogaster								
2	♀	44.7	26.4	10.8	9.6	2.5	1.7	4.4
	♂	26.2	26.6	16.1	17.0	7.3	3.1	3.7
W_8^F	♀	33.5	24.5	10.7	12.4	5.6	3.4	9.8
	♂	38.6	24.7	15.6	9.5	3.9	2.5	5.3
1	♀	40.2	25.6	8.3	11.1	5.2	3.3	6.4
	♂	31.6	18.4	12.3	15.9	9.3	6.0	6.5
E11	♀	37.6	15.8	6.7	12.0	9.4	7.0	11.5
	♂	36.2	12.7	7.3	13.2	13.3	7.8	9.5
D. simulans								
2	♀	44.1	24.6	9.6	12.7	4.3	1.2	3.4
	♂	43.2	18.7	9.9	16.0	4.7	3.9	3.8
3	♀	47.1	19.9	10.5	14.2	3.0	1.9	3.4
	♂	40.6	24.5	10.5	12.4	4.5	3.4	4.2
R7	♀	56.3	23.6	6.2	8.0	2.1	1.1	2.7
	♂	44.6	19.5	10.2	11.3	5.5	3.4	5.5
I_{13}^J	♀	34.9	24.9	16.8	15.5	3.9	1.3	2.6
	♂	26.7	24.6	16.9	18.4	7.2	3.8	2.5

The high F value for positions is not unexpected in view of previous experiments showing positive phototaxis in both species (see McDonald and Parsons, 1973). Of interest for this paper is the significant positions × species interaction, showing some difference in the overall spatial distribution of the species. Inspection of Fig. 1 shows that there is a general fall in percentages from positions 1 to 7 in both species but that the fall is more rapid in *D. simulans*, so that at positions 6 and 7 nearly double the percentage of *D. melanogaster* individuals are found compared to *D. simulans*. In other words, *D. simulans* is more dependent on the presence of light than *D. melanogaster*, as found previously.

Other significant components include the positions × sexes interaction and the positions × strains within-species interaction. The interaction with sexes probably reflects a somewhat higher proportion of females in positions 1 and 2, and thereafter the situation is largely reversed. In other words, females tend to be attracted slightly more to the light than are males. The positions × strains within-species interaction indicates some differences between strains within species. The triple interaction, positions × sexes × strains within species, is also significant.

More flies went to position 7 than position 6 in both species (Fig. 1), which is of interest since the light intensity at position 7 was 10 lux or half that of position 6. Examination of Table I shows that this situation occurs

Table II. Analyses of Variance of Phototactic Responses in *Drosophila melanogaster* and *Drosophila simulans*[a]

Source of variation	df	MS	Tested against which error	F	P
Positions	6	78,493.54	1	114.69	<0.01%
Positions × species	6	1,626.47	1	2.38	5%
Positions × sexes	6	964.32	2	3.62	1%
1. Positions × strains within species	36	684.37	3	6.32	<0.01%
Positions × species × sexes	6	118.08	2	0.44	N.S.
2. Positions × sexes × strains within species	36	266.70	3	2.46	<0.01%
3. Within	784	108.26			

[a] The within item consists of the variation between the eight replicates (df = 7) × 2 sexes × 8 strains × 7 positions = 784 df. A complete factorial analysis was carried out (with strains nested within species), and all items not shown in the table were not significant. A mixed-model analysis of variance was used, hence sources of variation were tested against different errors (see Snedecor and Cochran, 1967, p. 368).

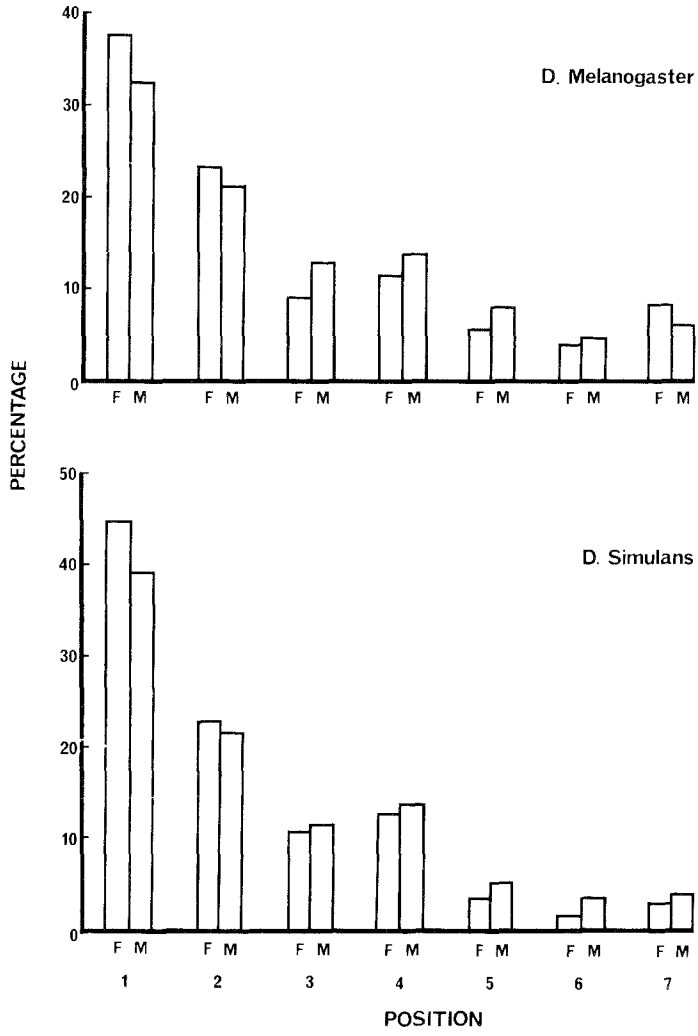

Fig. 1. Percentages of flies at each of the seven positions for the two species (summing over strains).

for all sexes and strains in *D. melanogaster*, and in *D. simulans* the same applies except for the males of two strains. It was also noted that flies at the bright end were very active compared to the flies at the dull end of the box. The flies at the dull end of the box usually just sat on the food dish and moved very little after getting there.

DISCUSSION

The results using this design for the strains tested are in qualititative agreement with those obtained using design class 1 (McDonald and Parsons, 1973) for the following reasons:

1. *D. simulans* as a species shows greater positive phototaxis than *D. melanogaster*.
2. Within species, there is heterogeneity between strains, as found by McDonald and Parsons (1973).

Heterogeneity between isofemale strains for quantitative behavioral traits seems a general phenomenon. For example, Parsons and Kaul (1967) found considerable differences in duration of copulation between strains of ST/ST and CH/CH karyotypes of *D. pseudoobscura* which were derived from the same population, as did Spiess *et al.* (1966) and Parsons and Kaul (1967) for traits essentially measuring mating speed. In *D. melanogaster*, Hosgood and Parsons (1967) came to similar conclusions for mating speed and duration of copulation using isofemale strains. Rockwell and Seiger (1973*b*) found heterogeneity for phototactic response using design class 2 in *D. pseudoobscura* and *D. persimilis*. The present results are therefore not surprising, especially in view of the now universal observation of heterogeneity between isofemale strains for quantitative traits (see Parsons, 1973*a,b,c*). Quite clearly, a different set of strains could give variant results—but the results are in *qualitative* agreement with previous cited work on *D. melanogaster* and *D. simulans*.

It can be concluded that for two of the basic designs for measuring phototaxis in *Drosophila* there is some agreement in the results for the two sibling species *D. melanogaster* and *D. simulans*. McDonald and Parsons (1973) used a light source parallel to the plane of movement, and in the design under discussion a light source perpendicular to the plane of movement was used. As Rockwell and Seiger (1973*a*) point out, both designs are measurements of a response to light but neither can be regarded as measuring exactly the same response. Although there is a basic agreement between the results for the two designs, it is difficult to make comparisons precisely. Quoting from Rockwell and Seiger (1973*a*),

> Phototaxis is a complex behavioral response to light that begins with the impingement of light on the photoreceptor and proceeds through a chain of events that culminates in the locomotion, or lack of locomotion, of the organism. Its measurement in the laboratory is dependent on the experimental design, which is a function of the research interest of the investigator.

This shows the type of dilemma that results from attempting to generalize results obtained on specific behavioral traits.

Referring to the observation of an association of phototaxis with locomotion, there is far less general activity at position 7 than at the bright end of the testing box. Some flies must be regarded as being negatively phototactic associated with a low level of general activity, although of course the flies are active enough to move from the inlet hole in the center of the apparatus to position 7. It would seem that such negatively phototactic flies may be quite common in natural populations since most strains tested showed more flies at position 7 than at position 6. Quite likely, such flies are genetically phototactically negative. The apparatus therefore may provide a method of selecting for phototactically negative flies in natural populations since we are dealing with only about 5% of the population. Breeding from these individuals would no doubt increase the frequency of negative phototaxis in subsequent generations. This possibility is being tested.

With reference to the quotation above and taking up one of the research interests of the investigator, the existence of variability within each of the two species is clear, as is the overall difference between the species. In this sense, the catalogue of behavioral/ecological differences found between the two species as listed in McDonald and Parsons (1973) should be consulted. The basic question to answer is, Do differences in phototaxis within and between the species have any evolutionary significance? Perhaps we cannot in the immediate future hope to aim for a result as definitive as found from exposing adults of the two species to a choice of media containing 0 and 9% alcohol (McKenzie and Parsons, 1972). Behaviorally, *D. melanogaster* had a small preference for oviposition on the alcoholic medium, while *D. simulans* showed a highly significant preference for the standard medium sites. This is associated with a high sensitivity of *D. simulans* to alcoholic media, and indeed in a vineyard and maturation cellar near Melbourne only *D. melanogaster* is found. Furthermore, during vintage, limited data indicate that *D. melanogaster* moves toward the cellar in a regular fashion and *D. simulans* moves away from it (McKenzie, 1974). Therefore, the distribution of the two species at vintage may be a function of their dispersal activities. Hence from laboratory and field experiments it can be concluded that alcohol in the environment allows *D. melanogaster* to extend its niche while the same is not true of *D. simulans*. Returning to phototaxis, extrapolation to the field situation of the results found so far in the laboratory, whereby *D. simulans* is more light dependent than *D. melanogaster*, has yet to be done. Until this can be done, the evolutionary significance of the difference must remain obscure. It should be noted that Grossfield (1971) suggested that the unique situation of *D. simulans*, as the cosmopolitan species with the greatest light dependence, may reside in its close relationship to *D. melanogaster* reflecting behavioral divergence from

it. But this does not give us the adaptive significance of the difference in nature, although there may well have been selection for behavioral divergence, as suggested by Grossfield. If it could be shown that differences in phototaxis lead to utilization of somewhat different resources for each species in nature, as shown for alcohol, then the evolutionary significance of the result would be clearer.

Evidence was presented and references were given in McDonald and Parsons (1973) showing that *D. melanogaster* could be regarded as occupying a broader niche than *D. simulans* for survival at extreme temperatures and for dependence on light. In the experiment under discussion, it was possible to investigate niche breadth by using the proportions p_i of the total number of flies at each of the seven positions in the apparatus and then calculating the niche breadth as

$$H = \sum_{i=1}^{N} p_i \log_e p_i$$

where N is the total number of positions (see MacArthur, 1965). H is large if the niche is broad and small if it is narrow. The results were as follows: *D. melanogaster* 1.68 (females), 1.77 (males), and *D. simulans* 1.31 (females), 1.64 (males), showing in both sexes that flies are more evenly distributed in the apparatus for *D. melanogaster*. Therefore, this species may have a broader niche for phototaxis than *D. simulans*, as might be expected, although not too much weight should be placed on these estimates because of the nonsignificant positions × species × sexes interaction in Table II.

ACKNOWLEDGMENTS

I am grateful to Mrs. R. L. McKenzie, who carried out the tedious observational work, to Dr. D. A. Hay for help with the calculations, and to the Australian Research Grants Committee for financial support.

REFERENCES

Grossfield, J. (1971). Geographic distribution and light-dependent behavior in *Drosophila*. *Proc. Natl. Acad. Sci.* **68**:2669–2673.

Grossfield, J. (1972). The use of behavioral mutants in biological control. *Behav. Genet.* **2**:311–319.

Hadler, N. M. (1964). Heritability and phototaxis in *Drosophila melanogaster*. *Genetics* **50**:1269–1277.

Hosgood, S. M. W., and Parsons, P. A. (1967). Genetic heterogeneity among the founders of laboratory populations of *Drosophila melanogaster*. II. Mating behaviour. *Aust. J. Biol. Sci.* **20**:1193–1203.

MacArthur, R. H. (1965). Patterns of species diversity. *Biol. Rev.* **40**:510–533.

McDonald, J., and Parsons, P. A. (1973). Dispersal activities of the sibling species *Drosophila melanogaster* and *Drosophila simulans*. *Behav. Genet.* **3**:293–301.

McKenzie, J. A. (1974). The distribution of vineyard populations of *Drosophila melanogaster* and *Drosophila simulans* during vintage and non-vintage periods. *Oecologia* **15**:1–16.

McKenzie, J. A., and Parsons, P. A. (1972). Alcohol tolerance: An ecological parameter in the relative success of *Drosophila melanogaster* and *Drosophila simulans*. *Oecologia* **10**:373–388.

Parsons, P. A. (1973*a*). *Behavioural and Ecological Genetics: A Study in Drosophila*, Clarendon Press, Oxford.

Parsons, P. A. (1973*b*). Genetics of resistance to environmental stresses in *Drosophila* populations. *Ann. Rev. Genet.* **7**:239–265.

Parsons, P. A. (1973*c*) Quantitative variation in natural populations. *Genetics* (in press).

Parsons, P. A., and Kaul, D. (1967). Variability within and between strains for mating behaviour parameters in *Drosophila pseudoobscura*. *Experientia* **23**:131–132.

Rockwell, R. F., and Seiger, M. B. (1973*a*). Phototaxis in *Drosophila:* A critical evaluation. *Am. Scientist* **61**:339–345.

Rockwell, R. F., and Seiger, M. B. (1973*b*). A comparative study of photoresponse in *Drosophila pseudoobscura* and *Drosophila persimilis*. *Behav. Genet.* **3**:163–174.

Snedecor, G. W., and Cochran, W. G. (1967). *Statistical Methods*, 6th ed., Iowa State University Press, Ames, Ia.

Spiess, E. B., Langer, B., and Speiss, L. D. (1966). Heterogamic mating control by gene arrangements in *Drosophila pseudoobscura*. *Genetics* **54**:1139–1149.