HERITABILITY AND PHOTOTAXIS IN *DROSOPHILA MELANOGASTER*

NORTIN M. HADLER

Department of Biology, Yale University, New Haven, Connecticut

Received June 22, 1964

FOR decades the ground base of genetics has been the acceptance of the principle that the appearance, morphology, and physiology of any plant or animal is causally related to that organism's genotype and its interaction with its environment. However, the possibility that the same viewpoint is equally applicable to the organism's behavior has only recently been explored. In fact, the demonstration that behavior is a function of two independent variables, the genotype and the environment, is in contradistinction to the historical tendency in behavioral science to assume homogeneity of behavioral expression over the individuals or species under investigation. It is increasingly apparent that the variability of behavior is as universal a fact as is genetic variability.

The behavior of *Drosophila melanogaster*, indeed of the order Diptera, which was classically stereotyped as negatively geotaxic and positively phototaxic, has recently been shown to be plastic. HIRSCH (1959) developed a "multiple unit classification maze" for the mass screening of *D. melanogaster* for geotaxis. This maze is essentially a physical realization of the mass screening scoring technique (HIRSCH and TRYON 1956) and affords an objective, automatic and reliable means of assessing the geotaxic response of populations of *D. melanogaster*. Using these mazes HIRSCH and his colleagues were able to demonstrate and measure the variability of the geotaxic response (HIRSCH 1959) and to assess the contribution of each chromosome to the behavioral response (ERLENMEYER-KIMLING, HIRSCH and WEISS 1962; HIRSCH and ERLENMEYER-KIMLING 1962).

Phototaxis in *Drosophila* is equally amenable to an experimental behavior-genetic approach (HADLER 1964; HIRSCH and BOUDREAU 1958; MÉDIONI 1963). In the present paper, selection and hybridization experiments are described leading to an analysis of the heritability of this behavior. By adhering to a definition of heritability in the restricted sense, the behavior, phototaxis, is shown to be defined by the experimental design.

MATERIALS AND METHODS

The two photomazes used in the present experiments have been described previously (HADLER 1964). They consist of an arithmetic progression of consecutive Y-units in a horizontal plane (Figure 1). The mazes are so constructed that when illuminated from above, the alleys are in darkness with the exception of the "light" arm of each Y-unit. A "Plexiglas" cone is inserted in each arm of a Y-unit to minimize retracing (HIRSCH 1959).

A population of 200 females and 200 males is introduced into the stem of the first Y-unit. In passing through the maze, each individual makes 15 consecutive light/dark choices. The animals emerge in 16 collecting tubes, each containing a plug of culture medium. The collecting

FIGURE 1.—Diagrammatic representation of the hexagonal structural unit of the photomazes. Alleys are formed by glueing black nylon hexagons (rubber in the case of Maze I) onto a sheet of lucite. Lucite cones are inserted in each arm of a Y as shown.

tube into which a fly emerges establishes how many light or dark choices it has made in passing through the maze. A fly emerging into Tube 0 has made 15 consecutive light choices; into Tube 1, 14 light choices and one dark choice; . . . into Tube 15, fifteen consecutive dark choices. In this fashion the population is screened and its distribution in the collecting tubes, described in terms of its mean (photoscore) and variance, is a measure of its phototaxic response.

Two photomazes are now being used for separate selection experiments. The mazes are not structurally identical. To increase the difference between the two mazes, a double-bulb circular fluorescent fixture illuminates the surface of Maze II, at about 300 apparent foot candles, a single bulb fixture illuminates the surface of Maze I.

It is necessary to maintain environmental controls in a behavioral study; it is even more important to describe as fully as possible the environment used to elicit the behavior under study. This point can not be overemphasized and will be dealt with in the DISCUSSION. Such factors as mechanical stimulation, rearing conditions, age at time of trial, temperature and humidity, light and the effects of gravity and diurnal rhythms are maintained constant during this experiment. For a detailed description of these controls, see HADLER 1964.

The selection and hybridization experiments described in this paper involved over 45,000 flies. A foundation population was established in 1961 in a population cage from equal numbers of Formosa, Syosset and Capetown strains of *D. melanogaster* provided through the generosity of PROFESSOR TH. DOBZHANSKY. A sample containing 200 males and 200 females was drawn from this wild-type population and screened through the given maze. The 60 females and 60 males from whom the strongest photopositive response had been elicited were mated to establish the "light" (Lt) line. This was effected in six half-pint culture bottles ranked 1 through 6. Ten males and ten females were allocated to each bottle in such a fashion that the bottle rank corresponded to the response intensity, i.e., the ten most photopositive pairs were mated in Bottle 1. The "dark" line (Dk) was established in similar fashion from the photonegative end of the wild-type distribution.

By this procedure separate "dark" and "light" lines were established for Maze I and for Maze II, i.e., four separate, noninterbreeding populations. In succeeding generations, samples of 200 females and 200 males were drawn from the culture bottles, starting with Bottle 1. These samples were screened through the appropriate maze, and the extreme 60 males and 60 females from each strain were mated.

RESULTS

Selection pressure was applied for 30 generations to produce highly photopositive and photonegative strains. For the results of the first 15 generations see HADLER 1964 (Table 1). The results of the later generations of selection are

TABLE 1
Selection data

		Maze I				Maze II			
		Female	Light Male	Female	Dark Male	Female	Light Male	Female	Dark Male
G17	$\bar{x}$	4.45	3.94	11.54	12.63	4.00	4.85	11.28	12.21
	σ^2	4.22	5.48	5.47	3.52	4.31	5.49	5.83	4.84
	N	222	204	115	96	201	176	186	174
G19	$\bar{x}$	2.83	3.11	12.19	12.61	3.95	5.14	10.68	11.52
	σ^2	3.70	4.98	3.78	3.61	3.91	5.09	5.77	4.92
	N	193	166	165	123	214	207	174	157
G20	$\bar{x}$	1.91	1.75	11.99	11.99	4.27	4.68	10.70	11.70
	σ^2	2.81	2.70	3.75	5.23	5.33	4.87	6.50	5.90
	N	207	198	212	177	210	195	202	179
G21	$\bar{x}$	2.11	1.94	11.71	12.11	3.39	4.11	10.57	10.99
	σ^2	3.17	3.65	5.35	4.50	4.75	4.46	4.01	4.14
	N	211	166	204	166	213	159	203	175
G22	$\bar{x}$	1.43	2.05	12.37	12.82	3.46	4.40	10.50	11.68
	σ^2	1.97	4.01	3.76	2.67	5.26	6.63	5.55	3.89
	N	227	188	213	198	211	197	201	186
G23	$\bar{x}$	2.27	2.00	11.39	12.03	2.71	2.58	11.90	12.17
	σ^2	3.39	3.14	4.90	5.63	3.79	2.87	5.20	4.16
	N	227	220	219	202	224	208	218	216
G24	$\bar{x}$	2.55	2.60	12.75	12.44	3.41	3.81	12.90	12.83
	σ^2	4.43	4.72	3.06	3.88	6.18	6.77	3.58	3.46
	N	212	198	217	207	226	215	125	129
G25	$\bar{x}$	1.94	1.81	12.71	12.65	3.08	3.50	12.10	12.55
	σ^2	2.72	2.72	3.23	2.78	4.62	5.57	4.11	3.79
	N	213	197	225	223	211	208	183	166
G26	$\bar{x}$	1.28	1.11	10.27	10.68	2.11	1.86	10.26	11.55
	σ^2	1.49	1.88	6.66	5.51	3.20	2.31	4.18	3.46
	N	221	208	215	199	200	200	218	194
G27	$\bar{x}$	1.61	1.45	11.87	11.84	2.14	2.20	11.56	11.49
	σ^2	2.55	2.55	4.74	5.94	2.96	3.11	5.05	5.68
	N	222	216	203	184	221	219	220	197
G28	$\bar{x}$	3.08	2.33	12.57	12.91	3.00	3.37	11.96	12.99
	σ^2	4.06	3.60	3.46	4.79	4.80	5.30	4.89	4.52
	N	197	187	167	162	211	218	212	199
G29	$\bar{x}$	3.45	2.83	11.68	11.77	3.93	4.06	12.38	12.51
	σ^2	3.93	4.31	3.36	3.26	6.44	6.01	2.84	2.97
	N	187	162	180	161	215	199	211	204
G30	$\bar{x}$	2.00	1.93	13.44	13.26	3.70	3.33	13.15	13.34
	σ^2	2.59	3.01	1.97	2.33	3.79	4.69	2.64	2.09
	N	189	200	224	190	209	200	234	209

Wild-type controls

	Maze I		Maze II	
	Female	Male	Female	Male
$\bar{x}$	7.88 $\pm$.58	8.70 $\pm$.63	9.97 $\pm$.52	10.46 $\pm$.48
σ^2	6.03 $\pm$ 1.31	6.55 $\pm$.99	7.72 $\pm$ 1.53	6.63 $\pm$.54

given in Table 1. Included in Table 1 are the results of nine wild-type control populations tested with Maze I and nine tested with Maze II. The averages of the means of these trials, averages of the variances and their respective standard errors are presented. The course of selection as indicated by the photoscore can be followed over 30 generations in Figure 2 for Mazes I and II. All strains have significantly diverged from the appropriate wild-type controls.

In Figure 3, cumulative variance is plotted as a function of generation of selection for Mazes I and II. These curves are meaningful *only* as a qualitative study of the change in variance as a function of the generation of selection and the

FIGURE 2.—Selection in Maze I and Maze II over 30 generations. The mean of the distribution of each sex of each strain is plotted as a function of generation of selection. The units of "photoscore" correspond to the numbers of the collecting tubes where the flies leave the maze. Flies emerging into Tube 0 have completed 15 consecutive light choices; into Tube 1, 14 light choices and one dark choice. Therefore, flies emerging into Tube 15 have completed 15 consecutive dark choices. In the Maze I plot, open circles represent females; closed circles, males. In the Maze II plot, open circles represent males, closed circles, females. Generations 16 and 18 were lost owing to incubator failure.

stability of the rate of change of variance. After G8, the curves for the Maze I strains are roughly parallel and decreasing in slope. This is to say that the size of the variance is decreasing as a function of generation of selection at a rate that is relatively constant over strain and sex. The curves for Maze II reveal a different phenomenon. There is little stability in the response of the variance to selection, particularly for the light strain. These curves qualitatively resemble those constructed from the data obtained during the first eight generations of selection in Maze I.

When the variance of the selected population approaches the standard error of the variance of the wild-type population, selection will reach a limit as the mazes no longer discriminate among individuals. The asymptotes thus defined (HADLER 1964) are now being strongly approached. In an ideal instrument, selection should proceed to isogenicity in each strain and sex, barring heterosis. That this is not the case was demonstrated by the effectiveness of reverse selection (started at G27) in forcing the photoscore and variance back toward the appropriate wild-type control.

In an isogenic strain, the observed variance is dependent solely on the environment. The wild-type variance is dependent on both the genome and the environ-

FIGURE 3.—Cumulative variance is plotted as a function of the generation of selection. The variances of the two missing generations (16 and 18) are approximated by averaging the variances of the two generations preceding and following the missing generation.

ment. Heritability (h^2) can be computed by the following formula based on the above two statements: $h^2 = (\sigma_o^2 - \sigma_g^2)/\sigma_o^2$ where σ_o^2 is the wild-type variance and σ_g^2 is the variance of an isogenic line.

The variances of the strains of G30 are approaching the limits of selection of the mazes. It follows that these variances are approaching the variances characteristic of isogenicity in these strains. It is therefore possible to make a conservative, lower-limit estimate of heritability. There is no statistically significant sexual dimorphism in behavior in any of the G30 strains. Since the variances of the Lt and Dk strains (sexes combined) for each maze are significantly different at the 5 percent confidence level, heritability estimates must be calculated separately for the two mazes. The following are the lower-limit estimates of heritability: Maze I Lt: $h^2 = .564$; Maze I Dk: $h^2 = .658$; Maze II Lt: $h^2 = .412$; Maze II Dk: $h^2 = .668$.

Table 2 presents the results of a preliminary hybridization analysis. Several trends are apparent in these data. In the reciprocal crosses, the offspring, particularly the daughters, of Lt females are characteristically more photopositive than the offspring derived from Dk females, in both mazes and in all hybridization generations. Thus the X chromosome appears to be implicated in the discussion of heritability. A significant increase is observed in the F_2 variances over the F_1 variances with no significant difference between the F_2 and F_3 variances. The observed trend in variance across the F_1 , F_2 and F_3 is expected

TABLE 2

Hybridization analysis: Statistical constants for F_1 , F_2 , and F_3

F No.	Maze	G*	Dk female $\times$ Lt male						Lt female $\times$ DK male					
			Female		Male		Female and male combined		Female		Male		Female and male combined	
			$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2
F_1	I	21	6.60	6.81	5.85	6.72	6.23	6.77	5.55	5.78	7.06	10.05	6.31	7.92
F_1	I	25	5.94	7.05	7.60	8.71	6.77	7.88	5.64	7.41	7.38	6.53	5.51	6.97
$\bar{x}$			6.27		6.73		6.50		5.60		6.22		5.91	
σ^2				6.93		7.72		7.32		6.59		8.28		7.45
F_1	I	21	5.59	12.03	6.12	13.68	5.86	12.86	...	...	...	...	...	...
F_2	I	25	6.96	8.99	6.70	10.00	6.83	9.49	4.85	9.79	6.11	9.95	5.48	9.87
$\bar{x}$			6.27		6.41		6.35		...		...		...	
σ^2				10.51		11.84		11.17	...		...		...	
F_3	I	25	9.07	8.76	9.61	8.36	9.34	8.56	8.55	10.29	8.79	9.81	8.67	10.15
F_1	II	21	6.89	7.71	9.14	6.47	8.02	7.09	6.81	6.61	8.39	8.03	7.60	7.32
F_1	II	25	6.27	12.20	7.79	11.94	7.03	11.07	6.07	8.64	5.96	6.98	6.02	7.81
$\bar{x}$			6.58		8.47		7.52		6.44		7.17		6.81	
σ^2				9.95		9.20		9.08		7.63		7.51		7.56
F_2	II	25	7.77	11.91	7.84	9.69	7.80	10.79	5.56	12.37	6.80	10.97	6.18	11.67
F_3	II	25	9.60	9.30	9.43	10.30	9.52	9.80	8.17	11.33	7.95	10.95	8.06	11.14

* G: Generation of selection at which cross was made.

(ERLENMEYER-KIMLING *et al.*, 1962; MATHER 1949) and tends to rule out cytoplasmic inheritance as a factor to consider in the discussion of heritability.

DISCUSSION

The degree to which the present selection experiment is effective depends upon the reliability of the phenotype as a measure of the genotype. If the environment is kept constant, any permanent change in the behavioral response of the population may be attributed to a change in its genetic composition. Both the extent to which selection has been effective, and the gradual and prolonged divergence of the strains demonstrate that a polygenic system is involved in the expression of phototactic behavior. The hybridization analysis suggests the involvement of the X chromosome in this polygenic system.

Heritability can be briefly defined as "the proportion of the trait variance in a specified population which is determined by the genotypic variation in that population" (FULLER and THOMPSON 1960, p. 62). If this definition is to be meaningful the concept of "trait variance" must be understood. Behavior is the observed response of an organism to a particular stimulus environment. The trait variance in the population is dependent both on the genotypic variability of that population and on the environmental variance. This dependence is described most succinctly by its effects on heritability. For instance, the same trait variance could be achieved by reducing the genotypic variability (as in the case of selection experiments) and increasing the environmental variance, or vice versa. In the first instance, heritability would be lowered, while in the second it would be increased. Moreover, if the same population were exposed to two different stimulus environments and different behavioral responses were elicited, there is no reason to assume that the genetic components of the two elicited responses are identical, i.e., involve the same components of the genome. This would be true even for the unusual case when the heritability estimate was the same for both responses.

The plasticity of expression of phototaxis in *D. melanogaster* as measured in Maze I and Maze II is apparent. That Maze I and Maze II represent different stimulus environments was demonstrated in the discussion of their structural differences and differences in illumination. There are probably microenvironmental differences as well. Therefore, one can not justifiably conclude that the behavioral responses elicited by the two mazes are the same. This statement is supported by the discussion of the cumulative variance curves (Figure 3). An analysis of the variances of the G30 strains in both mazes revealed a significant difference between the Lt and Dk strains of each maze. This is to say that the polygenic systems upon which photopositive and photonegative behavior in each maze are dependent are not (and may never be) quantitatively the same. As well, there is no reason to assume that they are qualitatively the same. (The results of the chromosome assay for genotaxis by J. HIRSCH and L. ERLENMEYER-KIMLING [1962] lead to comparable deductions.) In other words, no single behavior—"phototaxis"—is analyzed in this work. Rather four behaviors are under investigation: photopositive behavior in Maze I, photonegative behavior in Maze I, photopositive behavior in Maze II, and photonegative behavior in Maze

II. For this reason, a lower-limit estimate of the heritability of each behavior is given in RESULTS. Heritability is not a characteristic or correlate of a behavior *per se*. It is a characteristic of a particular population with respect to a particular behavior, in a given environment.

One common characteristic of research in experimental behavior genetics is the fact that results can not be quantitatively reproduced. Even if one were to use the same mazes and foundation population, two insurmountable obstacles would remain: the necessity of duplicating the temporal variations in the micro-environment and the error incurred in sampling populations. The reliability of the sampling technique can be estimated by the formula: $r = (\sigma_o^2 - \sigma_e^2) / \sigma_o^2$, where σ_o^2 is the wild-type variance and σ_e^2 is the standard error of the wild-type variance. For this experiment the reliability in sampling was about 85 percent.

The author gratefully acknowledges his indebtedness to PROFESSOR D. F. POULSON for valuable consultation and for providing laboratory space and facilities and to DRs. L. ERLNMEYER-KIMLING and DAVID SEEGAL for valuable consultation. This work was supported by National Science Foundation (URP) Awards administered through Yale University.

SUMMARY

Two different multiple Y-unit mazes, which permit the assessment of the mean and variance of the phototactic behavior in *Drosophila* populations, have been used for separate 30-generation selection experiments and hybridization analyses. The behavior is shown to be dependent upon a polygenic system in which the X chromosome is implicated.

Heritability is not a characteristic of a behavior *per se*, but of a particular population with respect to a particular behavior elicited by a particular stimulus environment. Therefore no one behavior—"phototaxis"—is analyzed with this approach. Four behaviors are studied: photopositive and photonegative behavior in Maze I and photopositive and photonegative behavior in Maze II. The lower-limit estimates of the heritability of these behaviors are 0.564, 0.658, 0.412 and 0.668, respectively.

LITERATURE CITED

- ERLENMEYER-KIMLING, L., J. HIRSCH, and J. M. WEISS, 1962 Studies in experimental behavior genetics. III. Selection and hybridization analyses of individual differences in the sign of geotaxis. *J. Comp. Physiol. Psychol.* **55**: 722-731.
- FULLER, J. L., and W. R. THOMPSON, 1960 *Behavior Genetics*. Wiley, New York.
- HADLER, N. M., 1964 Genetic influence on phototaxis in *Drosophila melanogaster*. *Biol. Bull.* **126**: 264-273.
- HIRSCH, J., 1959 Studies in experimental behavior genetics. II. Individual differences in geotaxis as a function of chromosome variation in synthesized *Drosophila* populations. *J. Comp. Physiol. Psychol.* **52**: 304-308.
- HIRSCH, J., and J. C. BOUDREAU, 1958 Studies in experimental behavior genetics. I. The Heritability of phototaxis in a population of *Drosophila melanogaster*. *J. Comp. Physiol. Psychol.* **51**: 647-651.
- HIRSCH, J., and L. ERLNMEYER-KIMLING, 1962 Studies in experimental behavior genetics. IV. Chromosome analyses for geotaxis. *J. Comp. Physiol. Psychol.* **55**: 732-739.

- HIRSCH, J., and R. C. TRYON, 1956 Mass screening and reliable individual measurements in the experimental behavior genetics of lower organisms. *Psychol. Bull.* **53**: 402-410.
- MATHER, K., 1949 *Biometrical Genetics*. Methuen, London, England.
- MÉDIONI, J., 1963 La variabilité des comportements taxiques. Ses principales conditions écologiques et organiques. *Ergeb. Biol.* **26**: 66-82.