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Positive and Negative Geotaxis: Sex-Linked Traits in *Drosophila pseudoobscura*

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Using a Hirsch classification maze, selection was made for positive and negative geotactic behaviors in three different strains of Drosophila pseudoobscura. *Hybridization studies were then carried out with flies from the diverged strains. The geotactic scores of the parents and F_1 flies indicate that both negative and positive geotactic behaviors in these strains are strongly influenced by genes in the X chromosome. Additional hybridization studies using flies from strains with similar phenotypes suggest that the diverged strains contain similar alleles and the number of loci in the X chromosomes responsible for these types of behavior is limited. The loci may be highly organized in the X chromosome.*

KEY WORDS: geotaxis maze; geotactic behavior; *Drosophila pseudoobscura*; behavior genetics; sex linkage.

INTRODUCTION

Geotactic behavior in *Drosophila* may be quantitatively described by using a Hirsch classification maze (Hirsch, 1959). Natural populations of *D. pseudoobscura* tend to be neutral with respect to gravity in these mazes (Dobzhansky and Spassky, 1969). Even though the heritability of geotaxis is low (Richmond, 1969), artificial selection for positive and negative geotactic behaviors leads to the creation of divergent geopositive and geonegative strains of flies within a few generations (Dobzhansky and Spassky, 1962). The suspension of selection results in a rapid return to geoneutrality, suggesting that the genes responsible for this behavior in this

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species are maintained in the heterozygous state in natural and laboratory populations by natural selection (Dobzhansky and Spassky, 1962, 1967).

Woolf (1972) reported the results of hybridizations between a highly geopositive strain (strain 32) and a slightly geonegative strain (strain 31) of *D. pseudoobscura* which had been created by Professor Theodosius Dobzhansky and his colleagues at Rockefeller University. The results showed that the majority of the genes giving positive geotactic behavior reside in the X chromosome. Females from strain 32 and their F_1 sons tended to show similar positive geotactic scores. Because a highly geonegative strain was not available at that time for hybridization studies, no definitive statement could be made concerning the location of the genes for geonegative behavior.

The present article reports the results of genetic studies of geotactic behavior in strains 31 and 32 of *D. pseudoobscura* which have undergone additional selection, resulting in highly negative and positive geotactic behaviors, and in two newly created pairs of divergent geopositive and geonegative lines of this same species. The purpose of these studies was to determine the mode of inheritance of geonegative behavior, to gain further information on the genetic mechanisms influencing geonegative and geopositive behaviors in this species, and to learn if genetic differences occur among strains with a similar behavioral phenotype.

MATERIALS AND METHODS

Three pairs of divergent geotactic strains of *D. pseudoobscura* were used in this study. All were created by using Hirsch classification mazes as selection devices. Strains 31 (geonegative) and 32 (geopositive) are descendants of the strains used by Woolf (1972). They were derived originally from a sample collected at Piñon Flats, Mt. San Jacinto, California. These strains have undergone continuous selection since 1971, when they arrived at Arizona State University. Strains 33 (geonegative) and 34 (geopositive) were derived from a base population consisting of flies collected at Mather, California, and a highly photonegative strain (strain 27) used by Woolf (1972), which was available in the laboratory at Arizona State University (Woolf, 1972). Strain 27 was derived from a sample collected at Piñon Flats, Mt. San Jacinto, California. The flies from Mather, California, were sent to Arizona State University by Dr. Wyatt Anderson. Strains 41 (geonegative) and 42 (geopositive) were derived from a sample collected at the McDonald Ranch, California, and sent to Arizona State University by Dr. Francisco Ayala. Strains 33, 34, 41, and 42 were geoneutral when the selection experiments were begun. All strains were highly divergent when hybridizations were made in January 1976.

Hybridizations were performed by placing 20 virgin females and 20 males in each of five different half-pint culture bottles containing standard cornmeal–molasses–agar medium with tegosept as the mold inhibitor. Females were allowed to lay eggs for 3 days in each culture bottle; the parents were then transferred to fresh culture bottles. All progeny were reared at 24 ± 1°C. Using this procedure, successive F_1 broods were available for testing in the mazes, allowing for replications of each test.

The geotaxis mazes used in these experiments have been described elsewhere (Hirsch, 1959; Dobzhansky and Spassky, 1967). Flies entering a maze make 15 consecutive choices either upward against gravity or downward toward gravity and emerge in one of 16 collecting tubes at the end of the maze. Flies which make all upward choices emerge in tube 1; those which make all downward choices emerge in tube 16. The number of flies in each collecting tube is used to compute the mean geotactic score. A mean of 8.5 is expected of geoneutral flies. A mean of 1.0 is the most extreme geonegative score; a mean of 16.0 is the most extreme geopositive score.

RESULTS AND DISCUSSION

Geotactic scores of each divergent strain of flies at the time of the hybridizations are shown in Table I. The geotactic scores of the F_1 flies resulting from reciprocal matings of flies from the diverged strains (31 × 32, 33 × 34, and 41 × 42) are also presented in Table I. In each case the geotactic scores of the F_1 males differ significantly from those of their sisters. F_1 females tend to exhibit geotactic behavior which is intermediate to the two parental strains, but F_1 males tend to behave similarly to their mothers. These findings demonstrate that the X chromosome is important for both negative and positive geotactic behavior in these strains of *D. pseudoobscura*. The data also give evidence for the influence of autosomal genes on both types of behavior; F_1 males have a slightly more geoneutral score than males from their mother's strain.

Polygenic traits are often characterized by genetic heterogeneity; identical phenotypes can be produced by different genotypes (Lerner, 1954, 1958). The availability of three different geopositive strains and three different geonegative strains all showing sex-linked inheritance presented an opportunity to determine whether each type of behavior is influenced by similar loci. If the same loci are involved, females which have an X chromosome from each of two different geopositive or two different geonegative strains should behave similarly to females from the parental strains. If the loci are different, these females should show a neutral behavior. It is likely that the geotactic scores of these females could be used as an indicator of the similarity of the loci.

Table I. Geotactic Scores of Geopositive and Geonegative Strains and F_1 Hybrids

	Females		Males		
Parental strains	$\bar{x}$ + SE	n	$\bar{x}$ + SE	n	H[a]
Strain N31	4.91 ± 0.24	321	4.63 ± 0.22	342	
Strain P32	14.37 ± 0.21	216	14.78 ± 0.12	218	
Strain N33	2.22 ± 0.12	337	3.17 ± 0.21	245	
Strain P34	15.47 ± 0.09	114	15.48 ± 0.09	181	
Strain N41	5.49 ± 0.27	258	4.68 ± 0.32	289	
Strain P42	14.87 ± 0.14	142	14.89 ± 0.11	176	
F_1 hybrids					
N31♀♀ × P32♂♂					
Replication 1	8.35 ± 0.24	274	4.98 ± 0.22	292	
Replication 2	7.69 ± 0.22	343	3.71 ± 0.18	301	
Replication 3	10.65 ± 0.24	206	6.48 ± 0.22	253	
Pooled	8.63 ± 0.14	823	4.97 ± 0.13	846	316.85[b]
P32♀♀ × N31♂♂					
Replication 1	9.94 ± 0.22	319	13.22 ± 0.19	277	
Replication 2	10.18 ± 0.22	309	12.06 ± 0.21	429	
Replication 3	10.06 ± 0.17	396	14.06 ± 0.15	260	
Pooled	10.06 ± 0.12	1024	12.93 ± 0.12	966	355.45[b]
N33♀♀ × P34♂♂					
Replication 1	9.74 ± 0.24	294	6.91 ± 0.38	119	
Replication 2	12.40 ± 0.20	251	6.45 ± 0.24	251	
Replication 3	11.68 ± 0.24	185	5.63 ± 0.36	132	
Pooled	11.14 ± 0.14	730	6.37 ± 0.17	502	335.99[b]
P34♀♀ × N33♂♂					
Replication 1	12.06 ± 0.18	338	13.62 ± 0.22	175	
Replication 2	10.98 ± 0.28	207	13.28 ± 0.17	323	
Pooled	11.64 ± 0.16	545	13.43 ± 0.14	498	113.31[b]
N41♀♀ × P42♂♂					
Replication 1	7.54 ± 0.32	135	4.79 ± 0.24	254	
Replication 2	9.15 ± 0.29	177	6.86 ± 0.34	190	
Replication 3	8.26 ± 0.27	166	5.98 ± 0.33	168	
Pooled	8.47 ± 0.17	478	5.76 ± 0.17	612	129.77[b]
P42♀♀ × N41♂♂					
Replication 1	9.16 ± 0.28	220	10.99 ± 0.29	181	
Replication 2	9.13 ± 0.30	184	10.91 ± 0.37	145	
Replication 3	11.89 ± 0.25	124	13.09 ± 0.30	102	
Pooled	9.79 ± 0.19	528	11.46 ± 0.19	428	43.90[b]

[a] H obtained from Kruskal-Wallis nonparametric test. Hypothesis: pooled mean of F_1 females is equal the pooled mean of F_1 males.

[b] $P < 0.001$.

Matings were made to produce females with each combination of the three geopositive or geonegative X chromosomes. The geotactic scores of flies from each strain at the time of these hybridizations and the geotactic scores of the F_1 females are presented in Table II. In all cases the pooled F_1 geotactic scores are similar to the scores for the parental strains, indicating a similarity of the loci.

The present study expands the earlier findings of Woolf (1972) with strains 31 and 32 in that geonegative as well as geopositive behavior shows strong sex linkage. When the earlier hybridizations were performed, strain 31 was only slightly geonegative and therefore the results yielded no

Table II. Geotactic Scores of Geopositive and Geonegative Strains, Female Hybrids of Geonegative Strains, and Female Hybrids of Geopositive Strains

	Females			Males	
Parental strains	$\bar{x}$ + SE	n		$\bar{x}$ + SE	n
Strain N31	1.85 ± 0.08	393		2.81 ± 0.07	361
Strain P32	15.17 ± 0.07	330		15.23 ± 0.08	312
Strain N33	3.45 ± 0.13	331		4.14 ± 0.18	379
Strain P34	15.22 ± 0.09	200		15.23 ± 0.09	286
Strain N41	4.45 ± 0.19	260		4.24 ± 0.21	229
Strain P42	15.11 ± 0.10	152		14.80 ± 0.11	274
Female hybrids of geonegative strains			Female hybrids of geopositive strains		
N33♀♀ × N31♂♂			P34♀♀ × P32♂♂		
Replication 1	1.93 ± 0.12	277	Replication 1	15.38 ± 0.09	88
Replication 2	2.84 ± 0.14	312	Replication 2	15.50 ± 0.08	114
Replication 3	4.22 ± 0.21	232	Replication 3	15.59 ± 0.08	110
Replication 4	3.34 ± 0.15	348	Replication 4	15.12 ± 0.09	171
Pooled	2.97 ± 0.08	1069	Pooled	15.50 ± 0.05	483
N41♀♀ × N33♂♂			P34♀♀ × P42♂♂		
Replication 1	4.78 ± 0.28	206	Replication 1	14.95 ± 0.10	214
Replication 2	4.78 ± 0.20	307	Replication 2	15.15 ± 0.09	218
Replication 3	3.74 ± 0.19	262	Replication 3	15.06 ± 0.11	199
Replication 4	4.26 ± 0.18	329	Replication 4	15.10 ± 0.08	289
Pooled	4.38 ± 0.10	1104	Pooled	15.10 ± 0.04	920
N31♀♀ × N41♂♂			P32♀♀ × P42♂♂		
Replication 1	3.56 ± 0.20	230	Replication 1	15.24 ± 0.13	75
Replication 2	3.45 ± 0.14	372	Replication 2	14.90 ± 0.11	183
Replication 3	4.46 ± 0.29	259	Replication 3	15.28 ± 0.15	154
Replication 4	3.22 ± 0.17	273	Pooled	15.04 ± 0.05	412
Pooled	3.76 ± 0.10	1134			

information on the genetics of this type of behavior in *D. pseudoobscura.* The additional period of selection in strain 31 has led to an increase in the frequency of geonegative alleles at the responsible loci in the X chromosome. Furthermore, we have demonstrated a sex-linked mode of inheritance for both geonegative and geopositive behavior in flies derived from two other laboratory populations of *D. pseudoobscura.* While both negative geotaxis and positive geotaxis show strong sex linkage, our results do not resolve whether the loci controlling positive geotactic behavior also control negative geotactic behavior by series of contrasted alleles. There is no reason to assume that the same loci control both types of behavior. Markow (1975) reported that in *D. melanogaster* negative phototactic behavior is controlled by loci in the X chromosome and positive phototactic behavior is controlled by loci in the autosomes.

It would be of interest to determine if geotactic behavior is strongly influenced by sex-linked genes throughout the geographic range of *D. pseudoobscura.* The studies reported here should be repeated using samples of flies from diverse populations of this species. It would also be of interest to know if genes controlling geotactic behavior are sex linked in closely related species of *D. pseudoobscura,* such as the sibling species *D. persimilis.* Geotactic behavior in *D. melanogaster* is largely controlled by genes in the autosomes (Hirsch and Erlenmeyer-Kimling, 1962; Pyle, 1975). Differences in the genetics of geotactic behavior among closely related species of *D. pseudoobscura* are not unexpected. Negative phototactic behavior which was shown to be sex linked in one strain of *D. melanogaster* (Markow, 1975) was shown to be autosomally inherited in the sibling species *D. simulans* (Markow and Smith, 1977).

The results of the study carried out by Woolf (1972) suggested that the bulk of the genes for positive geotactic behavior in strain 32 of *D. pseudoobscura* occur in a region of the X chromosome not readily separated by crossing over; however, he stated that his data did not rule out the possibility of the effect of a single gene. If a polygenic mode of inheritance is accepted for *D. pseudoobscura,* as proposed by Dobzhansky and Spassky (1962, 1967, 1969), it might be assumed that the responsible genes are highly organized in the X chromosome. A similar situation was described by Dubinin (1948) and discussed by Lerner (1954) for a polygenic system controlling extra wing veinlets in *D. melanogaster*; the responsible genes are clustered in two specific regions of chromosome 2. Genetic variation at the loci in these regions is maintained in natural populations by heterozygote superiority. Thoday (1961) found "major-effect loci" underlying high and low bristle number phenotypes in *D. melanogaster* and suggested that other polygenic traits may have a similar underlying genetic organization. Any genetic model for geotactic behavior in *D. pseudo-*

obscura should incorporate the observation in our study that all combinations of geopositive × geopositive matings and geonegative × geonegative matings produced progeny that were similar to the parental types. This phenomenon suggests that the number of loci is limited and the same alleles are present in the diverged strains with the same phenotype. It is unfortunate that precise linkage studies are not possible at the present time in *D. psuedoobscura.* Such studies could yield important information on the nature of the genetic mechanism responsible for geotactic behavior in this species.

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