4-1997

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Published in *Animal Behaviour* 53:4 (April 1997), pp. 853–864; doi: 10.1006/anbe.1996.0351

Submitted February 26, 1996; accepted July 16, 1996; published online May 25, 2002.

Y-chromosome Effects on *Drosophila* Geotaxis Interact with Genetic or Cytoplasmic Background

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Abstract

Previously, all of the major fruit fly, *Drosophila melanogaster*, chromosomes (I, II, and III) have been shown to be associated with geotaxis, but the Y chromosome has not. Using two methods (back-crossing and chromosome substitution), Y chromosomes from lines that have evolved stable, extreme expressions of geotaxis were placed into different genetic and cytoplasmic backgrounds to test the resulting males for geotaxis. The results of the back-crossing do not support the interpretation of Y-chromosome effects on geotaxis. These tests do not have sufficient statistical power, however, to detect small genetic effects. In the chromosome substitution experiment, the geotaxis-line Y chromosomes were placed into high- and low-selected lines, Canton-S and Champaign wild-type backgrounds. The results of the chromosome substitution experiment provide evidence for a Y-chromosome effect on geotaxis in selected geotaxis lines but not in wild-type stock, backgrounds. These results suggest that the Y chromosome has a small effect on geotaxis, whose detection depends on genetic and/or cytoplasmic background. The implications of these results are discussed for behavior-genetic analysis of *D. melanogaster* and for issues of statistical power in detecting small genetic effects.

It is inescapable that gravity is an environmental factor of paramount importance. Given its pervasive nature, we can sometimes fail to recognize gravity's vital role in fundamental

behavioral processes like orientation and the maintenance of posture and equilibrium in other behavior patterns such as flying, swimming, and walking (Horn 1985). Although the physiological basis of gravity sensing systems is fairly well understood in some species (Schwarzkopff 1964; Horn 1985), much remains to be learned about how animals relate to gravity. The ways in which animals (especially insects) orient and move with respect to gravity (i.e., geotaxis) can be considered to be evolutionarily important behaviors because they have the potential to affect habitat selection, foraging success, mating success, or other important aspects of life history.

Although heredity-geotaxis relations have been studied with the *Drosophila melanogaster* model system for more than 40 years (e.g., Hirsch & Tryon 1956; Stoltenberg & Hirsch 1996) our understanding is far from complete. Each of the three major chromosomes is correlated with geotaxis (Ricker & Hirsch 1988a, b). Stoltenberg et al. (1995) identified three allozyme markers (alcohol dehydrogenase, ADH; amylase, AMY; and 6-phosphogluconate dehydrogenase, PGD) associated with geotaxis in lines that have evolved stable, extreme expressions of that behavior (Ricker & Hirsch 1985). In an F_2 correlational analysis, the ADH-geotaxis association was maintained, but the AMY-geotaxis association was broken by meiosis; therefore, ADH is a marker for a gene correlate of geotaxis but AMY is not (the PGD-geotaxis association was not tested in the F_2 generation; Stoltenberg et al. 1995). Subsequent analysis of two lines derived from those F_2 generation hybrids, after 66 generations of potential recombination, provides further evidence for a gene correlate of geotaxis near the structural gene for ADH (Adh 2-50.1; Stoltenberg & Hirsch 1996).

Stoltenberg et al. (1995) also used hybrid correlational analysis to examine the nature of the association between geotaxis and mate preference reported by Lofdahl et al. (1992) and found that those two phenotypes are influenced by separate genetic systems. Hybrid correlational analysis is a valuable technique to study phenotype-phenotype, genotype-phenotype, or genotype-genotype correlations in any species where control of matings is possible.

Despite such progress, an important proportion of the *D. melanogaster* genome remains unstudied for heredity-geotaxis relations. Both the Y and fourth chromosomes have been, and continue to be, routinely ignored or systematically controlled in the search for genetic correlates of *Drosophila* behaviors, including geotaxis.

At least three factors appear to be responsible for this lack of interest in Y chromosome–behavior relationships. First, the Y chromosome is heterochromatic; that is, the chromosome is usually found in a condensed, inactive state (see Williamson 1976 for a review of the genetics of the Y chromosome). Second, although the Y chromosome constitutes about 12% of the *D. melanogaster* genome (Pimpinelli et al. 1978), relatively few Y chromosome genes have been located. The Y chromosome contains genes that are essential for sperm maturation (Gatti & Pimpinelli 1983) and repetitive structural genes for the 18S and 28S ribosomal subunits (reviewed by Ritossa 1976). Third, few studies have examined Y chromosome–behavior relations with sufficient statistical power to detect small genetic effects. For example, in a frequently cited study, Safir (1920) made qualitative observations of the sexual behavior of 54 males lacking Y chromosomes (i.e., XO) and reported it to be "normal" (page 478). Such small samples can often reliably detect large genetic effects; since it is generally accepted, however, that behavior often has polygenic correlates, as we have

already shown for geotaxis (Ricker & Hirsch 1988a, b), it is a reasonable inference that "small" genetic effects will be more common.

This report describes our attempts to detect Y-chromosome effects on geotaxis. We were motivated to conduct these experiments by the history of ignoring potential Y chromosome–behavior relations and by geotaxis score data (Ricker 1984) as part of a large-scale hybridization study to assess heredity-geotaxis relations. The geotaxis score distributions of Ricker's back-crosses suggested a Y-chromosome effect but were not conclusive. Here we describe two experiments designed to take advantage of the *D. melanogaster* model system to increase statistical power sufficiently to detect Y-chromosome effects on geotaxis.

General Methods

Requests for samples of the selected geotaxis lines should be addressed to Jerry Hirsch.

Subjects

Flies were cultured in 10.0 × 3.5-cm diameter plastic vials with yeasted Instant Drosophila Medium (Carolina Biological Supply, Inc., Burlington, North Carolina). Recurring bacterial infection that threatened the health of stocks kept in our laboratory prompted the use of antibiotic solutions to hydrate the instant medium. Throughout stock-keeping, the food was hydrated with one of three antibiotic solutions on a rotating schedule so that we used different antibiotic solutions in consecutive generations to avoid the evolution of bacterial resistance (Stoltenberg 1995). In a given experiment, however, a single antibiotic was used to avoid potential antibiotic effects on geotaxis. We etherized, collected, and sexed flies for testing from parent vials that housed five pairs of adults for 5 days to standardize larval density. When virgins were required, we collected flies within 5 h of eclosion. All vials were kept in a controlled environmental chamber at 25 ± 2°C and 50 ± 5% relative humidity and with a 16:8 h light:dark cycle with lights on at about 0830 hours.

Geotaxis Testing

The geotaxis mazes consist of a series of choice points at which individual flies have the option to walk either with the pull of gravity (down; positive geotaxis) or against the pull of gravity (up; negative geotaxis; Hirsch 1959). Sixteen terminal collection tubes are positioned at the end of each maze and are numbered to indicate the number of up choices made by each fly finishing in that tube. Individuals making all up choices finish in tube 15, and those making all down choices finish in tube 0. Flies making both up and down choices assort themselves into the collection tube that reflects the number of up choices.

We stored flies to be tested in same-sex vials that contained up to 115 individuals. To allow time for the flies to recover from the effects of anaesthesia, we began testing on the second day following the final collection. Most subjects were 2–5 days old at the start of testing, although we occasionally used flies up to 8 days old. Since we tested only males and were not concerned about their virginity, we cleared all adults from the parent vials and collected newly eclosed males 2 or 3 days later.

We began testing by loading a squad of about 200 males into a 19.0 × 1.25-cm diameter vinyl start tube which we then laid horizontally near the maze. Following a 30-min rest

period, we gently attached the start tube to the maze. Flies were given 24 h to walk through the maze. Testing began before 1100 hours. We tested 14,673 flies for geotaxis.

Statistical Analyses

In most cases, geotaxis score distributions are sufficiently non-normal to warrant using nonparametric statistics. We compared geotaxis score distributions using a Monte Carlo C × R (column × row) contingency table analysis (W. Engels, unpublished software). Such a test enables one to ask whether the rows and columns of a given contingency table are independent of one another and is preferable to a chi-square test when the expected number contained in some cells of the table is below five. The test randomly picks tables with the same marginal sums as the observed data and asks whether each differs from the expected value as much or more than the observed data. *P*-values are the ratio of the number of trials where the random tables differ as much or more than the observed data to the total number of trials (W. Engels, unpublished software).

Experiment 1: Backcrosses

By using backcrossing, we produced flies for geotaxis testing that, in each successive generation, had increasingly similar genetic backgrounds and a Y chromosome originating from either the high- or the low-selected geotaxis line. In each of seven successive generations, we crossed hybrid males back to females from either the high- or the low-selected geotaxis line. Furthermore, in each of seven successive generations, we tested about 200 males from each of the four backcross types to assess the efficacy of backcrossing to reduce genetic material from the nonrecurring line and to measure the Y-chromosome effect on geotaxis.

Method

Mating scheme

We set up 10 parent vials for each geotaxis line at generation 777, and again at each of the next eight generations, to provide virgin females for the backcrossing procedure. Unless otherwise indicated, 10 parent vials were used for each cross at each generation.

To begin, we made reciprocal crosses of the high- and low-geotaxis lines (high females × low males [HL], and low females × high males [LH]). The resulting F_1 generation male progeny were then crossed back to virgin females from the geotaxis lines to establish four backcross sublines (i.e., H × HL, H × LH, L × HL, L × LH). We collected B_1 generation males and mated them back to virgin females from the selected geotaxis lines in the parent vials from which B_2 generation males were to be collected. We repeated this basic procedure to prepare backcross males for geotaxis testing and for further backcrossing.

After a given number of generations of such backcrossing, the proportion of males with given genotypes can be estimated. In each backcross generation, four genotypes are present (Fig. 1). In the first backcross generation (B_1), the four genotypes should occur at equal frequency, assuming equal viability. Since recombination in male *D. melanogaster* is very rare, intact chromosomes are passed from father to progeny (Ashburner 1989). During

successive backcross generations, the proportions of each genotype change (Table I). The proportion of males with the genotype having the X chromosome and autosomes from the line of the recurring female (genotype A in Fig. 1, Table I) increases in frequency each generation, while the proportions of the other three genotypes (B, C , and D) decrease. After seven successive generations of backcrossing, we expect about 98% of the individuals from generation B_7 to have the genotype that, in a sense, reconstitutes that of the selected line from which the recurring female parents are drawn.

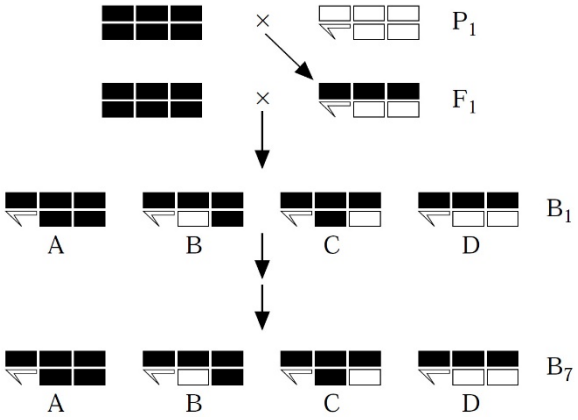

Figure 1. Schematic representation of a backcross in which females from line 1 (black bars) were crossed to males (with hook-shaped Y chromosome) from line 2 (white bars). Their F_1 generation male progeny were then crossed back to females from line 1. In the B_1 generation, males of four genotypic classes were produced in equal proportions. Successive generations of backcrossing to line 1 females increased the proportion of type A males and decreased the proportions of types B, C, and D males to B_7 (Table I).

Table I. Estimated genotype proportions changing over generations of backcrossing

	Genotype			
Backcross generation	A	B	C	D
1	0.250	0.250	0.250	0.250
2	0.563	0.188	0.188	0.063
3	0.766	0.109	0.109	0.016
4	0.879	0.059	0.059	0.004
5	0.938	0.030	0.030	0.001
6	0.969	0.015	0.015	< 0.001
7	0.984	0.008	0.008	< 0.001

Let n = backcross generation, according to equation: pAn = pAn-1 + 1/2pBn-1 + 1/2pCn-1 + 1/4pDn-1; pBn = 1/2pBn-1 + 1/4pDn-1; pCn = 1/2Cn-1 + 1/4pDn-1; pDn = 1/4pDn-1; where pA1 = pB1 = pC1 = pD1 = 0.250. Rows may not sum to 1.000 because of rounding errors.

Procedure

Throughout this experiment, we first collected virgin females and young males for breeding before we collected males to be tested for geotaxis, so that we would have enough flies

to set up 10 parent vials to produce the next generation. Because we were testing only males for geotaxis, we were not concerned with their virginity and thus collected them on the second day after the collection of males for the parent vials.

Backcross males that were progeny of females from the same geotaxis line but had Y chromosomes from either the low- or high-geotaxis line (e.g., L × HL and L × LH) were tested separately but in the same maze to facilitate comparison of the resulting geotaxis score distributions (Erlenmeyer-Kimling et al. 1962; Hirsch & Ksander 1969). At each of seven successive backcross generations (B_1–B_7), we tested one sample of about 200 males from each of the four backcross types (i.e., L × HL, L × LH, H × HL, H × LH). We also tested about 200 males from each selected line at G_{777} and from each reciprocal F_1 generation hybrid (i.e., LH and HL).

Results and Discussion

The results of the geotaxis tests of successive backcross generations do not lend themselves to unambiguous interpretation. In some generations, comparisons of sublines with the same genetic and cytoplasmic backgrounds, but different Y chromosomes, were consistent with the interpretation of a Y-chromosome effect on geotaxis, but in other comparisons the evidence was not consistent with such an interpretation. In generation B7, when we expected genetic noise to be nearly eliminated, we did not detect a Y-chromosome effect on geotaxis.

Figure 2 presents the geotaxis score distributions of seven consecutive generations of backcrossing (a–g, respectively) to low-line females (L × HL and L × LH). To control the experiment-wise error rate, we considered only *P*-values of < 0.007 (0.05/7) to be statistically significant. In three of seven backcross generations (B_2, B_3, B_4), the geotaxis score distributions of males with different Y chromosomes were significantly different. Only in generation B_2, however, was the direction of the difference consistent with the line of origin of the Y chromosome (Fig. 2b).

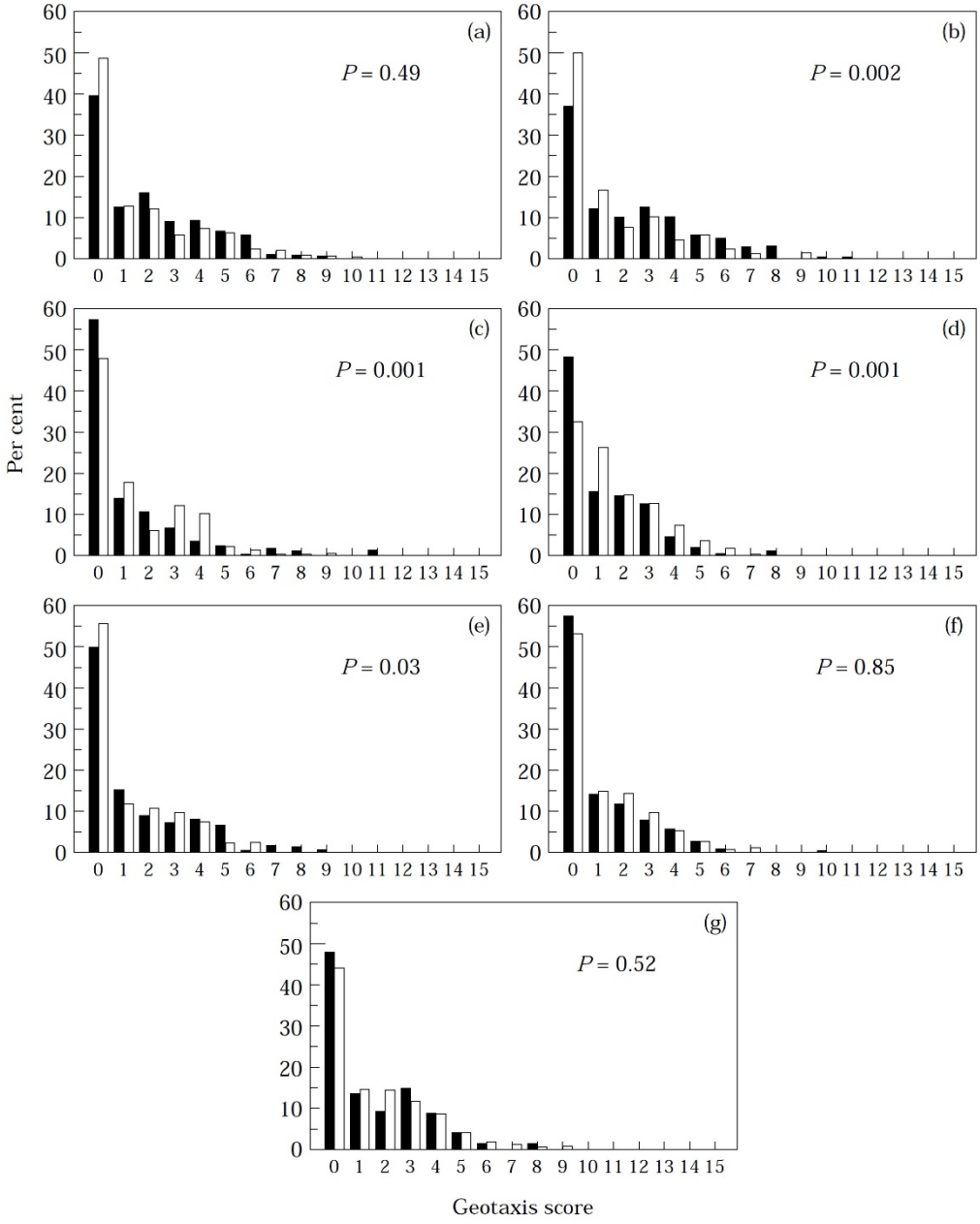

Figure 2. (a–g) Geotaxis score distributions of seven respective generations of backcrosses (B_1–B_7) to females from the low-geotaxis line. Black bars represent males with Y chromosomes from the high-line (i.e., L × LH); white bars represent males with Y chromosomes from the low-line (i.e., L × HL).

Figure 3 presents the geotaxis score distributions of seven consecutive generations of backcrossing (a–g, respectively) to high line females (H × HL and H × LH). At no point did

the geotaxis score distributions of males with different Y chromosomes differ sufficiently to reach the $P = 0.007$ level.

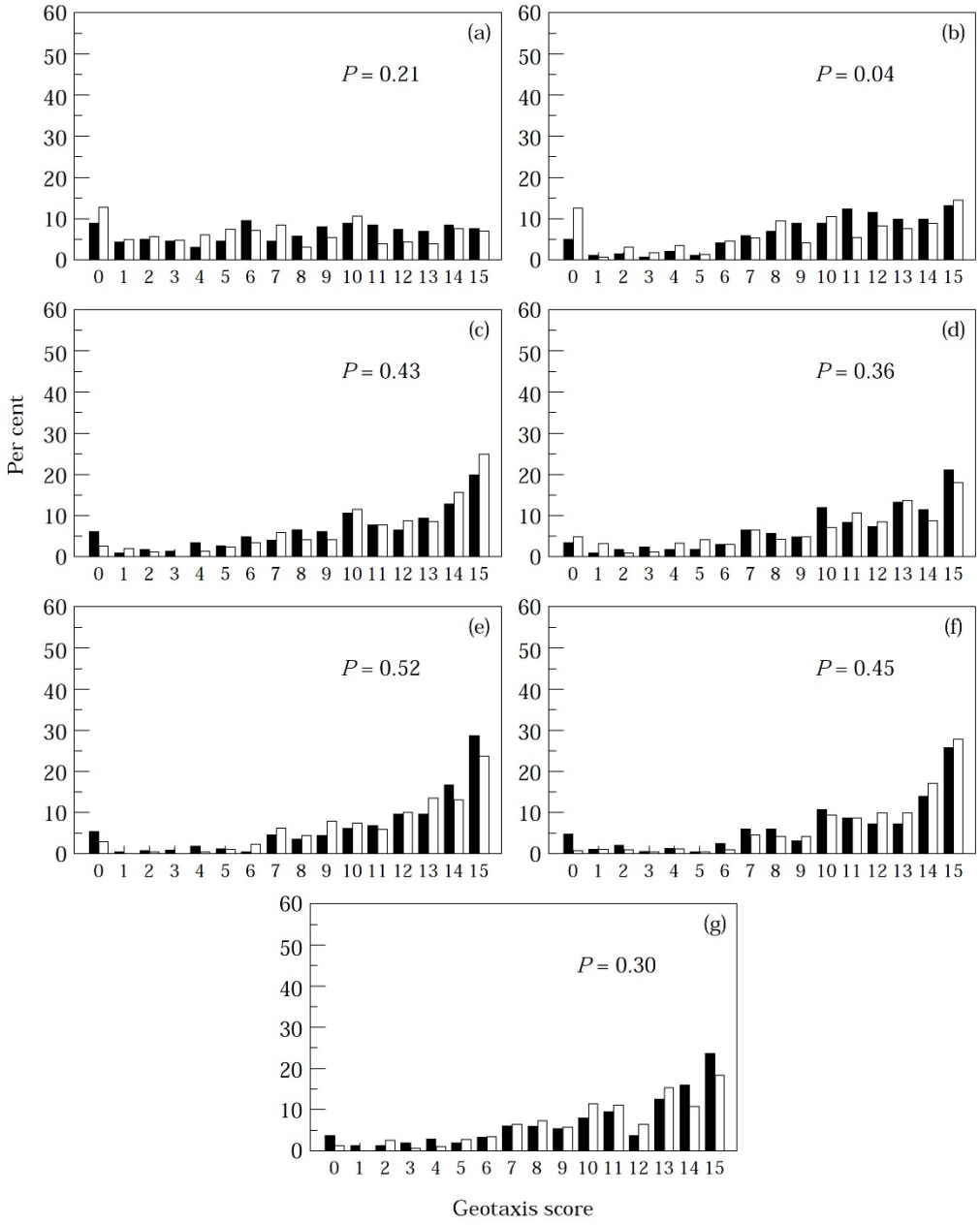

Figure 3. (a–g) Geotaxis score distributions of seven respective generations of backcrosses (B_1–B_7) to females from the high-geotaxis line. Black bars represent males with Y chromosomes from the high-line (i.e., H × LH); white bars represent males with Y chromosomes from the low-line (i.e., H × HL).

Comparisons of geotaxis score distributions of males with Y chromosomes from different geotaxis lines across seven generations of backcrosses did not consistently support the interpretation that the Y chromosome affects geotaxis. Interpretations of the backcross results should be made with some caution, however. Two features idiosyncratic to this design make such results difficult to explain.

First, we were not able to verify the frequencies of the genotypes present across the experiment. By assuming that the four genotypes are equally viable and represented proportionately throughout the study, we estimated their frequency each generation. These estimates cannot be empirically confirmed. It is possible that biased genotype frequencies could provide results similar to those observed. Evidence for residual genetic material from the nonrecurring line can be seen by comparing the geotaxis score means and variances of the backcross sublines at generation B_7 with those of the selected lines (Table II). Mean geotaxis scores of both high-backcross sublines (H × HL = 11.04, H × LH = 11.11) appear to be lower than the mean score of the high-geotaxis line at G_{777} (12.69). The variances of the high-backcross sublines (H × HL = 11.67, H × LH = 15.81) appear to be larger than the variance of the high-geotaxis line (9.38). Mean geotaxis scores of both low-backcross sublines (L × HL = 1.53, L × LH = 1.42) appear to be higher than the mean score of the low-geotaxis line (1.13). The variances of the low-backcross sublines (L × HL = 3.35, L × LH = 3.04) appear to be larger than the variance of the low-geotaxis line (2.82). Such a pattern suggests that residual autosomal material from the nonrecurring line was present in the backcross sublines, adding to genetic noise and making it more difficult to detect small Y-chromosome effects.

Table II. Geotaxis score summary statistics over backcross generations

Backcross generation	Mean	Variance	*N*
H × LH			
1	8.13	21.75	214
2	10.24	15.50	218
3	10.39	18.88	227
4	10.92	15.12	223
5	11.66	16.21	233
6	11.06	17.09	216
7	11.11	15.81	227
H × HL			
1	7.02	23.11	211
2	9.00	24.18	223
3	11.34	14.23	227
4	10.29	18.59	224
5	11.56	12.15	212
6	12.08	9.86	233
7	11.04	11.67	223
L × LH			
1	1.92	4.44	226
2	2.24	5.94	208
3	1.23	4.08	230
4	1.27	2.66	149
5	1.49	4.07	182
6	1.01	2.31	236
7	1.42	3.04	231
L × HL			
1	1.63	4.70	224
2	1.48	4.17	220
3	1.43	3.33	196
4	1.58	2.62	226
5	1.17	2.28	228
6	1.11	2.26	236
7	1.53	3.35	227

Second, in each generation we were able to collect only enough individuals for a single maze run per type. Thus, our sample sizes were necessarily constrained to about 200 for each type in each generation. Although 200 can be considered a rather large sample, it may not be large enough to provide the statistical power necessary to detect small effects. For a contingency table test with $df = 16$ and $\alpha = 0.05$ and an effect size = 0.20, $N_1 + N_2 = 933$ would be required for 99% power (Cohen 1988).

To remedy these two shortcomings of the backcross study and to control for the fourth chromosome, we used some of the most powerful and productive techniques available to Drosophilists. We describe the resulting chromosome substitution study next.

Experiment 2: Chromosome Substitution into Geotaxis Lines and Wild-Type Backgrounds

The results of experiment 1 do not support the interpretation that the Y chromosome is associated with geotactic performance in *D. melanogaster*. The experiment lacked the statistical

power to detect small effects; however, an improved test of such an association would reduce or eliminate residual genetic noise, thereby providing a more straightforward test of Y chromosomes from different geotaxis lines on the same genetic and cytoplasmic background. Such improvements would also make it possible to generate very large samples, increasing the statistical power of the tests so that small effects could be reliably detected.

The power of *Drosophila* genetics is brought to bear in the design of experiment 2. We used balancer stocks, with dominant morphological markers on the second, third, and fourth chromosomes, to track genetic material in a breeding scheme designed to produce flies with a common genetic and cytoplasmic background (from (a) high- or (b) low-geotaxis line or (c) Canton-S or (d) Champaign wild-type) but differing in Y chromosomes coming from the two geotaxis lines. We established such lines and compared their geotaxis score distributions.

Method

Subjects

We used flies from the high- and low-geotaxis lines at generation 782, from Canton-S and Champaign wild-type stocks, and from three balancer stocks. Flies in each balancer stock have an easily scored morphological marker, which results from a mutation that is dominant and homozygous lethal, so that flies with one copy of a mutation show the mutant phenotype, and those with two copies of a given mutation do not survive to adulthood. Such a system allows the experimenter to identify the origin of a fly's chromosomes in a breeding scheme. Progeny from a cross of a marker fly to a fly wild-type for that trait are structurally heterokaryotypic for the chromosome containing the marker. In a controlled breeding situation, the presence or absence of the dominant marker enables one to infer the origin of that chromosome's homologue. The marker on the second chromosome was Curly of Oster (CyO), a commonly used mutation that results in noticeably upturned wings. The stock CyO/Bc Elp (abbreviated CyO, hereafter) was provided by David Lampe (University of Illinois). The marker on the third chromosome was Stubble (Sb), which results in a phenotype where scutellar and sternopleural bristles are shorter and thicker than wild-type. The stock TM3, Sb/w (abbreviated Sb hereafter) was provided by the Bowling Green Stock Center. The fourth chromosome marker is *Cubitus interruptus* (Ci^D), which results in a wing vein (L4) that fails to reach the distal end of the wing, as it does in wild-type. The stock Bt^D/Ci^D (abbreviated Ci hereafter) was provided by the Bloomington Stock Center (see Lindsley & Zimm 1992 for descriptions of the balancer stocks).

Mating scheme

We first prepared females that were structurally heterokaryotypic at each of the second, third, and fourth chromosome balancer chromosomes (genotype 1i/1j; 2i/CyO; 3i/Sb; 4i/Ci, where 1, 2, 3, and 4 refer to the X, second, third, and fourth chromosomes, respectively, and i and j refer to nonisogenic homologous chromosomes) by first crossing flies from the CyO stock to flies from the Sb stock (G_1; Fig. 4). At G_2 we crossed their progeny (2i/CyO; 3i/Sb) to flies from the Ci stock. Then, to introduce the selected line Y chromosomes, we mated males from the geotaxis lines (1S/YS; 2Si/2Sj; 3Si/3Sj; 4Si/4Sj, where S refers to

chromosomes from either the high- or low-geotaxis line) to triple-marked females (1i/1j; 2i/CyO; 3i/Sb; 4i/Ci, (G_3)). At G_4 we mated 1i/YS; 2Si/CyO; 3Si/Sb; 4Si/Ci males to "background" females (B1i/B1j; B2i/B2j; B3i/B3j; B4i/B4j, where B refers to chromosomes from either the high- or low-geotaxis line, Canton-S, or Champaign stocks). At G_5, when the background females were from the same selected line as the Y chromosome (i.e., HY_H or LY_L) we collected males that were wild-type for all three mutations (B1i/YS; B2i/B2j; B3i/B3j; B4i/B4j) and mated them to females from that background stock to establish Y chromosome lines (HY_H and LY_L). In all other cases at G5, we mated triple-marked males (1Bi/YS; 2Bi/CyO; 3Bi/Sb; 4Bi/Ci) to females with the same background as in G_4. At G_6 we collected males that were wild type for all three mutations and mated them to females from the appropriate background stock to establish permanent Y chromosome lines (i.e., HY_L, LY_H , C-SY_H, C-SY_L, ChY_H, and ChY_L).

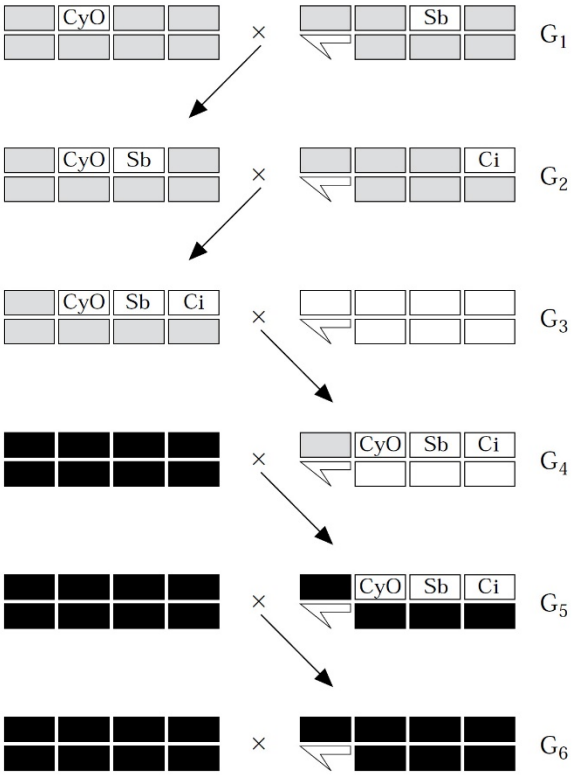

Figure 4. Schematic representation of the chromosome substitution mating scheme. Tinted and labeled (e.g., CyO) bars represent chromosomes from the balancer stocks. Open bars represent chromosomes from a selected geotaxis line (i.e., high or low). Shaded bars represent chromosomes from a background line (i.e., high- or low-geotaxis, or Canton-S, or Champaign wild-type).

Procedure

The procedure for setting up and maintaining parent vials is described in experiment 1. In some cases, however, owing to the low frequency of individuals with the desired phenotype (i.e., with all three mutations), fewer than five pairs of parents were used to set up parent vials. This was the case only during the mating regimen. During collection of subjects for geotaxis testing, parent vials were set up with five pairs of parents in each, as described earlier.

Since only males were tested for geotaxis, young flies were used (i.e., nonvirgins). As in experiment 1, to control maze effects, types of flies whose geotaxis score distributions were to be compared were run separately but in the same maze.

Results and Discussion

The geotaxis score distributions of the lines with background (X chromosome, autosomes, and cytoplasm) from the high line (i.e., HY_H and HY_L) were significantly different from each other ($P = 0.007$, $df = 15$; Fig. 5a). Visual inspection of the histograms indicates that the difference is primarily because a greater proportion of flies scoring 15 (i.e., 15 up choices) had Y chromosomes from the high line (HY_H) than had Y chromosomes from the low line (HY_L). Thus, the direction of the difference between the geotaxis score distributions of these two sublines is consistent with the line of origin of the Y chromosomes.

The geotaxis score distributions of the lines with background from the low line (i.e., LY_H and LY_L) are significantly different from each other ($P = 0.002$, $df = 15$; Fig. 5b). Visual inspection of the histograms indicates that the difference is primarily because a greater proportion of flies scoring 0 (i.e., 15 down choices) had Y chromosomes from the low line (LY_L) than had Y chromosomes from the high line (LY_H). Thus, the direction of the difference between the geotaxis score distributions of these two sublines is consistent with the line of origin of their Y chromosomes.

The geotaxis score distributions of the lines with background from the Canton-S line (i.e., C-S Y_H and C-S Y_L) were not significantly different from each other ($P = 0.61$, $df = 15$; Fig. 5c). We did not detect a Y-chromosome effect on geotaxis when Y chromosomes from the selected geotaxis lines were placed in Canton-S background.

The geotaxis score distributions of the lines with background from the Champaign line (i.e., Ch Y_H and Ch Y_L) were not significantly different from each other ($P = 0.11$, $df = 15$; Fig. 5d). We did not detect a Y-chromosome effect on geotaxis when Y chromosomes from the selected geotaxis lines are placed in Champaign background.

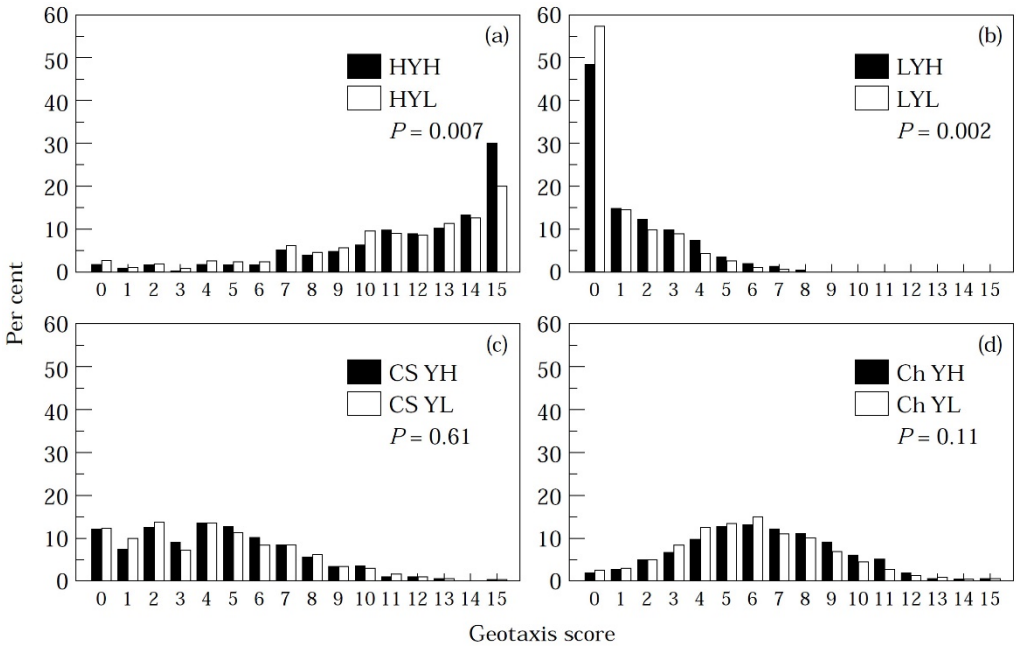

Figure 5. Geotaxis score distributions of lines with Y chromosomes from the high- (black bars) and low- (white bars) selected geotaxis lines in genetic and cytoplasmic background from (a) high-geotaxis, (b) low-geotaxis, (c) Canton-S wild-type, and (d) Champaign wild-type lines.

Mean geotaxis scores from the four sublines were associated with the line of origin of the Y chromosome in both selected line backgrounds, and in wild-type backgrounds (Table III). Although the mean geotaxis scores were in the direction consistent with the interpretation of a Y-chromosome effect on geotaxis regardless of background, in only the selected line background were the score distributions significantly different.

Table III. Geotaxis score summary statistics for flies with high- (Y_H) or low- (Y_L) geotaxis line Y chromosomes in different genetic and cytoplasmic backgrounds

Background	Mean	Variance	*N*
High			
Y_H	11.76	12.59	825
Y_L	10.95	14.65	866
Low			
Y_H	1.43	3.40	914
Y_L	1.06	2.52	882
Canton-S			
Y_H	4.40	9.09	1048
Y_L	4.23	9.31	1069
Champaign			
Y_H	6.47	8.75	1083
Y_L	6.00	8.34	1045

These results suggest that genes on the *D. melanogaster* Y chromosome are associated with geotactic behavior whose detection depends on genetic and/or cytoplasmic background. These results also suggest that further efforts to identify gene correlates of geotaxis ought to consider the Y chromosome. In addition, these results suggest that other Y chromosome–behavior correlates may be detected if experiments are conducted with sufficient power to detect small effects.

General Discussion

In this study we found evidence for a Y-chromosome effect on geotaxis in *D. melanogaster* whose detection depends on genetic and/or cytoplasmic background. The results of experiment 1, where we performed seven consecutive generations of backcrosses to females from the high- and low-selected geotaxis lines, do not support the interpretation of a Y-chromosome effect on geotaxis. The statistical power of those tests is not sufficient to detect small genetic effects, however. By improving our experimental design, i.e., by reducing genetic noise and increasing sample sizes, we detected a Y-chromosome effect on geotaxis in genetic and cytoplasmic background from the selected lines but not in background from two wild-type stocks. Thus, we have confirmed what appeared to us as a strong suggestion of a Y-chromosome effect on geotaxis (Ricker 1984).

If we assume that the Y chromosome contributes a small but consistent effect on geotaxis in concert with other parts of the genome, we can attempt to interpret the seemingly inconsistent results of this study. It is likely that little genetic variation relevant to geotaxis remains in the high- and low-selected geotaxis lines. Repeated attempts at reverse selection for geotaxis support this interpretation (Ricker & Hirsch 1985, 1988a, b; Stoltenberg et al. 1994). In addition, we recently reported (Stoltenberg et al. 1995) that we were unable to detect any allozyme variation within either selected geotaxis line. The selected lines may be fixed for alternative alleles at loci associated with geotaxis. In such homogeneous genetic backgrounds, small genetic effects may be more easily detected than in heterogeneous backgrounds. The genetic backgrounds of the backcrosses and the Canton-S and Champaign wild-type stocks are likely to be more variable at loci associated with geotaxis than are the selected line backgrounds. Small Y-chromosome effects may be, in a sense, drowned out by genetic variation associated with geotaxis in the backcrosses and in wild-type stocks.

These results do not enable us to determine whether detection of the Y-chromosome effect on geotaxis depends on genetic background or cytoplasmic background. There is abundant evidence that geotaxis is influenced by autosomal genes (Ricker & Hirsch 1988b), but to date there is no evidence that geotaxis is influenced by cytoplasm (Ricker & Hirsch 1988a). It is a reasonable inference that detecting the Y-chromosome effect on geotaxis depends on autosomal background; however, further studies are necessary for confirmation.

The present study is an empirical demonstration of the efficacy of the application of available techniques to increase the statistical power of tests for small genetic effects. By eliminating trait-relevant genetic variation and by increasing sample sizes in experiment 2, we achieved the statistical power necessary to detect small Y-chromosome effects on geotaxis that had heretofore gone undetected. Chromosome substitution techniques are

not widely available in species other than *D. melanogaster*; however, it is often quite possible to increase sample sizes. Studies that fail to detect specific genetic correlates of behavior should be interpreted with caution, and the statistical power of the tests used should be considered. Whenever possible, behavior-genetic analyses should be planned to maximize the probability of detecting small genetic effects by consulting tables that provide estimates of statistical power for given sample sizes, effect sizes, and alpha levels (e.g., Cohen 1988). It is also important to recognize that detecting interaction effects requires much greater power than does the detection of main effects in complex experimental designs (e.g., ANOVA; see Wahlsten 1990). Statistical power is one of the most important yet least understood concepts in behavioral science (Cohen 1990).

We are aware of only one other study that provides evidence for a Y-chromosome effect on behavior in *D. melanogaster*. Aslund et al. (1978) reported that males differing in the number of Y chromosomes (i.e., XO and XYY) were inferior to males with the usual Y-chromosome complement (i.e., XY) in aspects of mating ability. Our study appears to be the first to provide evidence of a Y chromosome–behavior association in *D. melanogaster* males with the usual Y-chromosome complement.

Fry et al. (1995) reported evidence for Y-linked mutations affecting abdominal bristle number in lines of *D. melanogaster* divergently selected on that trait. It may be that these mutations were at the *bobbed* (*bb*) locus, a well-studied Y-chromosome gene that affects bristle traits; however, Fry et al. were not able to identify the locus (or loci) involved. Few other studies have provided strong evidence of Y-chromosome effects on quantitative characters in *D. melanogaster* (Williamson 1976).

Evidence for a Y-chromosome effect on behavior has been found in mice, *Mus musculus*, perhaps the only other species where the body of empirical behavior-genetic analyses rivals that of *D. melanogaster*. The Y chromosome in mice has an effect on intermale aggression that depends on the autosomal background, the maternal environment, and the opponent's genotype (Carlier et al. 1991). Such a complex Y-chromosome effect is similar to the Y-chromosome effect on geotaxis described here, in that it is mediated by other factors. Roubertoux et al. (l994) recently localized the Y-chromosome effect on intermale aggression to a region of the mouse Y chromosome that recombines with the X chromosome at male meiosis. Recombination between the X and Y chromosomes can occur in *D. melanogaster*, but it is rare (reviewed in Williamson & Parker 1976). Further studies should be performed to localize the Y chromosome correlates of geotaxis in *D. melanogaster*.

The results of this study strongly indicate that the Y chromosome should not be ignored in the behavior-genetic analysis of *D. melanogaster*. In addition, such studies should be designed to have sufficient statistical power to detect small genetic effects that may depend on genetic, cytoplasmic, and where appropriate, maternal effects.

Acknowledgments – Thanks to Imelda Huerta for her invaluable assistance in backcrossing and to William Engels, University of Wisconsin, Madison, for supplying valuable 1988 Monte Carlo analysis software. This study was supported by the University of Illinois Foundation. This article is based on a dissertation by S.F.S. submitted to the University of Illinois at Urbana-Champaign in partial fulfillment of the requirements of the doctoral degree in psychology, in which the primary data are available.

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