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Autosomal and Maternal Effects on Pupation Behavior in *Drosophila melanogaster*

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This study demonstrates the importance of using a complete set of 16 reciprocal crosses (F_1, backcrosses, and F_2) to thoroughly investigate both genetic and nongenetic influences on patterns of inheritance of larval pupation behavior in Drosophila melanogaster. *Larvae derived from natural populations show significant variation in pupal height, defined as the distance a larva pupates above the feeding substrate. Differences in the distance a larva pupates from fruit in nature is known to affect the fitness of* Drosophila *populations. In this study the heredity of pupal height is analyzed by performing crosses between high- and low-pupating strains. We found that the inheritance of pupal height fit a classical additive polygenic model of inheritance, with intermediate F_1 pupal heights and greater variances in the F_2 generation. In addition, a significant maternal effect was also found by analyzing the reciprocal backcrosses. Progeny with low-pupating mothers had lower pupation heights than those with low-pupating fathers. Similarly, progeny with high-pupating mothers tended to have higher pupal heights than those with high-pupating fathers. This maternal effect was not attributable to strain differences in permanent cytoplasmic factors, sex chromosomes, or developmental time. Finally, we speculate upon the environmental conditions under which a transient maternal effect on pupation behavior would be expected to evolve in natural populations.*

KEY WORDS: *Drosophila melanogaster*; pupation behavior; maternal effect; heredity; natural selection.

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INTRODUCTION

Our previous work with larval pupation behavior has shown (1) genetic variation for differences in pupal height, in both laboratory (Sokolowski and Hansell, 1983) and natural populations (Sokolowski, 1985); (2) that differences in pupal height are influenced additively by both the second and the third pair of autosomes, with the second pair having three times the effect of the third (Bauer and Sokolowski, 1985); and (3) that differences in pupal survivorship can be related to the distance a larva pupates from the food in wet versus dry soil (Sokolowski, 1985).

The purpose of the present study was to elucidate further the genetic architecture of pupal height behavior by performing crosses between strains differing in this behavior, thereby enabling us to test for both chromosomal and nonchromosomal inheritance. *Drosophila* pupation behavior is of interest from an evolutionary perspective. In a heterogeneous environment the choice of a suitable pupation site is important to the successful emergence of the adult fly (de Souza *et al.*, 1970; Sokolowski, 1985; Schnebel and Grossfield, 1986).

The expression of a behavioral trait, a phenotype, is the result of both genotypic and environmental factors. For conceptual purposes we subdivide environmental factors into internal and external influences on the phenotype. External influences on a behavioral phenotype include all nongenetic influences on an organism (for example, moisture and temperature). Internal influences include the effect of the maternal parent on her offspring. A maternal effect is defined here as any influence a mother has on her offspring other than through the direct contribution of her chromosomes.

In mammals such as mice the mother can influence her offspring during three stages of early development: the period of oogenesis, the intrauterine period, and the postnatal period. During oogenesis the mother contributes materials to the egg cytoplasm for early embryonic development. The contribution of cytoplasm from the male parent is considered negligible (Ehrman and Parsons, 1981).

Two types of maternally derived nonchromosomal cytoplasmic factors, each showing a different pattern of inheritance, can be identified. The first are permanent cytoplasmic factors and the second are transient maternal factors. Permanent cytoplasmic factors include structures such as mitochondria which are transmitted over many generations (Wahlsten, 1979). In contrast, transient maternal factors dissipate over generations. Transient maternal factors can influence the progeny during oogenesis through maternal gene products, during the intrauterine period through the placenta, and during the postnatal period through parental care. The

mother has little time to influence an egg during the intrauterine period. A *Drosophila* female can lay 8–10 eggs in 20 min at 25°C: the egg may spend only 2–2.5 min in the uterus (Sonnenblick, 1950). The *Drosophila* egg is not physiologically dependent after oviposition. A *Drosophila* female exhibits the most basic form of parental care providing for her offspring's needs by her choice of oviposition site. For example, the number of eggs laid by a female at a resource limited site can influence the development of her offspring by the level of crowding at the site.

Grun (1976) reviews examples of permanent cytoplasmic effects in *Drosophila*. Luning (1983) used successive backcrosses to identify both permanent cytoplasmic and transient maternal effects on recombination rate in inbred *D. melanogaster*. Several mutants which exhibit transient maternal effects in *Drosophila* have been isolated (Spurway, 1948; Kaplan *et al.*, 1970; Baker, 1973; Mohler, 1977). In the case of the *rudimentary* mutant, offspring show abnormal embryogenesis due to a defect in pyrimidine biosynthesis in the mother (Okada *et al.*, 1974). In *grandchildless*, abnormalities in the formation of the offspring's gonads result in sterility (Mahowald *et al.*, 1979).

When biotic factors such as larval density are not controlled, a crossing analysis can show a maternal effect on the pattern of inheritance which has arisen due to differences in rearing conditions. An example of this is a study by Hay (1972a,b) on *D. melanogaster* adult preening behavior. He found a positive correlation between culture bottle density and preening behavior. Strain differences in culture bottle density result from strain differences in factors such as fecundity and egg hatchability. These factors are partly controlled by the maternal genotype. It was therefore not surprising to observe a maternal effect on preening behavior since strains with different culture bottle densities were compared.

Maternal effects on behavioral traits are not common in the behavior-genetics literature (Ehrman and Parsons, 1981). Rarely are experiments designed to distinguish between chromosomal and nonchromosomal factors influencing heredity. To separate maternal factors from genetic factors, it is important to perform F_1, backcross, and F_2 crosses between strains in a reciprocal manner (Wahlsten, 1979). The crosses required to detect the effects of transient maternal effects, permanent cytoplasmic effects, autosomes, and sex chromosomes are shown in Table I (modified from Wahlsten, 1979). Comparisons are made in a stepwise manner between crosses that are identical in three of four factors so that the unconfounded effect of the fourth factor can be determined. When the effect of one factor can be ruled out, the effect of another factor is then tested. We use this technique to measure the inheritance of larval pupation behavior in *D. melanogaster*.

Table I. Crosses Used to Separate Effects of the Autosomes, Transient Maternal Factors, Permanent Cytoplasmic Factors, and Sex Chromosomes[a]

Cross No.	Mother		Father	Autosomes	Transient maternal factors	Permanent cytoplasmic factors	Sex chromosomes		
							Female, XX	Male X	Male Y
Parental strains[b]									
1	L	×	L	L	L	L	LL	L	L
2	H	×	H	H	H	H	HH	H	H
F_1 generation[b]									
3	L	×	H	F_1	L	L	F_1	L	H
4	H	×	L	F_1	H	H	F_1	H	L
Backcrosses[c]									
5	L	×	LH	B_L	L	L	LL	L	H
6	L	×	HL	B_L	L	L	F_1	L	L
7	H	×	LH	B_H	H	H	F_1	H	H
8	H	×	HL	B_H	H	H	HH	H	L
9	LH	×	L	B_L	F_1	L	LL or F_1	L or H	L
10	HL	×	L	B_L	F_1	H	LL or F_1	L or H	L
11	LH	×	H	B_H	F_1	L	HH or F_1	L or H	H
12	HL	×	H	B_H	F_1	H	HH or F_1	L or H	H
F_2 generation[d]									
13	LH	×	LH	F_2	F_1	L	F_2	L or H	H
14	LH	×	HL	F_2	F_1	L	F_2	L or H	L
15	HL	×	LH	F_2	F_1	H	F_2	L or H	H
16	HL	×	HL	F_2	F_1	H	F_2	L or H	L

[a] L represents a low-pupating parent. H represents a high parent and LH or HL represents an F_1 parent. Female parents are always written first. F_1, F_2, B_L, and B_H indicate the distributions of chromosomes and transient maternal factors characteristic of an F_1 hybrid, an F_2 hybrid, a backcross to a low parental and a backcross to a high parental, respectively. Table modified from Wahlsten (1979).

[b] For females without the Y chromosomes, the comparison of 3 and 4 separates the effects of maternal factors from chromosomal factors. Also, comparing 1 and 3 as well as 2 and 4 separates embryonic chromosomal factors from maternal factors. For males, however, the Y chromosome and cytoplasmic factors are confounded.

[c] Effects of Y chromosomes for males and X chromosome for females: 5 versus 6; 7 versus 8. Effects of permanent cytoplasmic factors: 9 versus 10; 11 versus 12. Effects of transient maternal factors: for females, 5 or 6 versus 9; 7 or 8 versus 12. Effects of embryonic autosomes: 1 versus 6; 2 versus 7.

[d] Effects of Y chromosome for males: 13 versus 14; 15 versus 16. Effects of permanent cytoplasmic factors: 13 versus 15; 14 versus 16.

METHODS

Strains

Bauer and Sokolowski (1985) described the balanced lethal chromosome technique used to make *D. melanogaster* strains collected from nature isogenic for the second and third pairs of chromosomes. Two of the resultant strains, called B1B1 and B15B15, were used in the present study. Larvae of B1B1 pupate significantly higher than those of B15B15. The pupal heights of crosses between these two strains were tested as described below. The offspring of all the crosses (parental, F_1, backcross, and F_2) were bred and tested simultaneously to avoid the effects of random fluctuations of the environment on differences in pupation behavior between days. The parents of each of the crosses described in Table I were between 5 and 14 days old when mated. All strains, progeny of crosses, and test vials (described below) were maintained under standard conditions. Standard conditions consisted of a temperature of 24 ± 1°C, a relative humidity of 60%, uniform overhead illumination, and a photocycle of 12 h of light followed by 12 h of darkness, with lights on at 0800 h.

Test Procedure

The test procedure for measuring pupal height is described in detail by Bauer and Sokolowski (1985). For each of the crosses, 10 newly hatched larvae (±1.75 h in age) were placed into each of 10 vials (2 cm in diameter and 11 cm in height) using a dissecting probe. Each vial contained 5 ml of a dead yeast–agar medium. Larvae were placed on the central surface of the medium; the medium was not punctured with the probe. Vials were stoppered with standard-size cotton balls, placed in the outside longitudinal rows of test tube racks, and incubated under standard conditions. The positions of the 10 vials containing 10 larvae for each of the 16 crosses (1600 larvae in total) were completely randomized within and between test tube racks and the racks were positioned randomly in the incubator. After pupation the distance from the surface of the medium to a point between the two anterior spiracles of each pupa was measured. An average of 86% of the larvae reached the pupal stage per cross. Pupae were sexed as described by Bauer and Sokolowski (1985). The number and sex of emerging adults were also recorded daily. In 15 of 16 crosses the sex ratio was not significantly different from 1:1 (chi-square, $P > 0.50$). A rough estimate of developmental time was made for each of the 16 crosses by counting the number of male and female adult flies emerging daily.

Statistical Analyses

Contrast analyses of variances (ANOVAs) using SAS (1985) general linear models procedure were performed to determine the significance of the autosomes, transient maternal effects, permanent cytoplasmic effects, and/or sex chromosomes to differences in pupation behavior. Table II shows the a priori planned comparisons (Hays, 1981) that were chosen with the aid of Table I. Three ANOVAs were performed, one for each of the groups listed below, so that crosses with homogeneous variances were compared in each ANOVA. The variation within crosses between vials was included in all error estimates. Results of the ANOVAs for the female and male data are found in Tables IV and V, respectively.

1. *Parental and F_1 Generations.* The first group of comparisons included the parental strains and F_1 generations. This group tested (a) whether the parental strains were different from each other, (b) whether there were reciprocal differences in the F_1 generation, and (c) whether the F_1 generation showed dominance.
2. *Backcrosses.* A number of comparisons were made using the reciprocal backcrosses.
   (a) The effects of permanent cytoplasmic factors were tested.
   (b) The effects of transient maternal factors were also tested.
      (i) The initial comparison tested whether pupal heights of offspring from a low parental mother crossed to an F_1 father (L × LH or L× HL) were significantly different from those of an F_1 mother crossed to a low parental father (LH × L or HL × L).
      (ii) It also tested whether a high parental mother crossed to an F_1 father (H × LH or H × HL) was different from an F_1 mother crossed to a high father (LH × H or HL × H).
   (c) Sex linkage was also tested in the backcrosses.
      (i) The difference between a high and a low parental X chromosome (in the female data) or Y chromosome (in the male data) was tested in a low background (i.e., all other factors came from the low parental strain).
      (ii) The difference between a high and a low parental X chromosome (in the female data) or Y chromosome (in the male data) was tested in a high background (i.e., all other factors came from the high parental strain).
3. *F_2 Generation.* In the F_2 generation the effects of (a) the Y chromosome, (b) permanent cytoplasmic factors, and (c) the interaction between the Y chromosome and permanent cytoplasmic factors were tested in males; the effects of permanent cytoplasmic factors were tested in females.

Table II. Comparisons Used to Determine Effects of Autosomes, Transient Maternal Factors, Permanent Cytoplasmic Effects, and Sex Chromosomes[a]

	Comparison, cross No.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Parental and F_1 generations																
(a) Parentals	1	−1	0	0	—	—	—	—	—	—	—	—	—	—	—	—
(b) Reciprocal F_1	0	0	1	−1	—	—	—	—	—	—	—	—	—	—	—	—
(c) Dominance	1	1	−1	−1	—	—	—	—	—	—	—	—	—	—	—	—
2. Backcrosses																
(a) Permanent cytoplasm	—	—	—	—	0	0	0	0	1	−1	1	−1	—	—	—	—
(b) Reciprocal crosses for transient maternal effects																
(i) F_1 and low parentals ($F_1 \times L$ vs. $L \times F_1$)	—	—	—	—	1	1	0	0	−1	−1	0	0	—	—	—	—
(ii) F_1 and high parentals ($F_1 \times H$ vs. $H \times F_1$)	—	—	—	—	0	0	1	1	0	0	−1	−1	—	—	—	—
(c) High versus low X (♀'s) or Y (♂'s) chromosome in																
(i) Low background	—	—	—	—	1	−1	0	0	0	0	0	0	—	—	—	—
(ii) High background	—	—	—	—	0	0	1	−1	0	0	0	0	—	—	—	—
3. F_2 generation																
(a) Y chromosome (♂'s)	—	—	—	—	—	—	—	—	—	—	—	—	1	−1	1	−1
(b) Permanent cytoplasm (♀'s or ♂'s)	—	—	—	—	—	—	—	—	—	—	—	—	1	1	−1	−1
(c) Interaction between Y chromosome and permanent cytoplasm (♂'s)	—	—	—	—	—	—	—	—	—	—	—	—	1	−1	−1	1

[a] Dashes indicate crosses that were not used for a comparison. The symbol ♀'s or ♂'s indicates whether the female and/or male data were used to test the effect. See Table I for which crosses each cross number represents. See Materials and Methods for further explanation.

RESULTS

Effect of Autosomes on Pupal Height

The mean pupal heights, sample variances (s^2), and sample sizes (N) are shown in Table III for female and male progeny for each of the 16 crosses. Frequency distributions (Fig. 1 for the female and Fig. 2 for the male data) were constructed to examine the variance about the mean pupal height for each of the crosses. Visual inspection of these graphs indicated that the pattern of inheritance of pupal height followed that of a classical additive polygenic model. The F_1 generation (which were, presumably, mostly heterozygous for the trait measured) was intermediate to the two parentals (which were isogenic for their second and third pairs of chromosomes). The F_2 generation (the segregating generation) showed greater variances than the F_1 generation.

The data were normalized by using a natural logarithmic transformation (log) which reduced the positive correlation between the means

Table III. Mean Pupal Height, Variance, and Number of Pupae Measured for Female and Male Data from Crosses Between Two *D. melanogaster* Strains[a]

Cross, ♀ × ♂	Mean pupal height (cm)					
	Females			Males		
	Mean	s^2	N	Mean	s^2	N
Parentals						
1. L × L	1.0	0.15	37	1.0	0.24	35
2. H × H	2.2	0.36	49	2.5	0.73	40
F_1 generation						
3. L × H	1.3	0.17	55	1.6	0.27	42
4. H × L	1.5	0.26	53	1.7	0.32	43
Backcrosses						
5. L × LH	1.2	0.21	39	1.2	0.23	41
6. L × HL	1.3	0.20	47	1.4	0.26	41
7. H × LH	3.1	0.86	39	3.2	1.20	45
8. H × HL	2.0	0.44	53	2.1	0.39	44
9. LH × L	2.4	0.95	46	2.4	0.64	45
10. HL × L	2.0	0.58	43	2.3	0.95	49
11. LH × H	1.8	0.61	50	2.0	0.54	30
12. HL × H	2.0	0.88	52	2.3	0.99	36
F_2 generation						
13. LH × LH	1.9	0.84	41	2.0	1.52	27
14. LH × HL	1.6	0.28	42	1.7	0.44	34
15. HL × LH	1.7	0.75	49	2.0	0.69	41
16. HL × HL	2.2	1.08	44	2.3	0.84	42

[a] L represents a low-pupating parent, H represents a high parent, and LH or HL represents an F_1 parent.

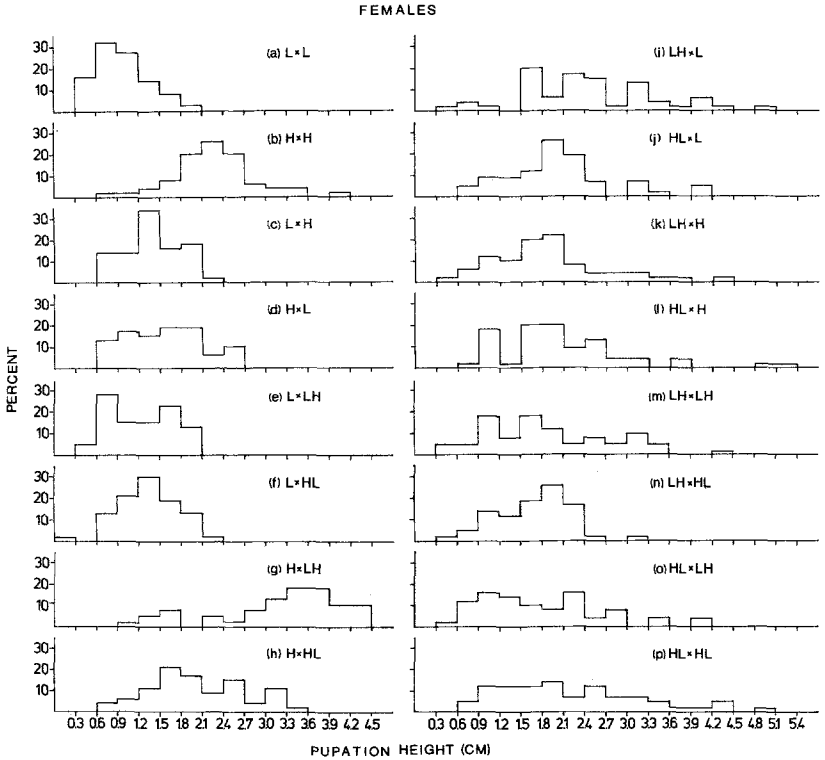

Fig. 1. The frequency distributions of the female progeny for crosses between the low and the high pupal height strains of *D. melanogaster*. L represents a low-pupating parent, H represents a high parent, and LH and HL represent an F_1 parent with the maternal contribution listed first; for example, LH means an F_1 with low mother cross high father. (a,b) the two parental strains; (c,d) the two F_1 generations; (e–j) the eight backcrosses; (m–p) are the four F_2 generations.

and the variances. The correlation between the mean pupal height and the variance before the log transformation was highly significant in the female ($r = 0.75$, $P < 0.001$) and male ($r = 0.71$, $P < 0.001$) data. all statistical analyses were performed on the log-transformed data.

The log transformation was successful in equalizing the variances for the two parental and F_1 crosses. The F_2 crosses still showed a greater variance than the F_1 crosses; this greater variance is consistent with the interpretation of an additive polygenic model for this trait. The ANOVA on the parental and F_1 crosses showed a significant difference in the parentals but no reciprocal or dominance effects. In both F_1 crosses the mean pupal heights were intermediate to the parental pupal height scores,

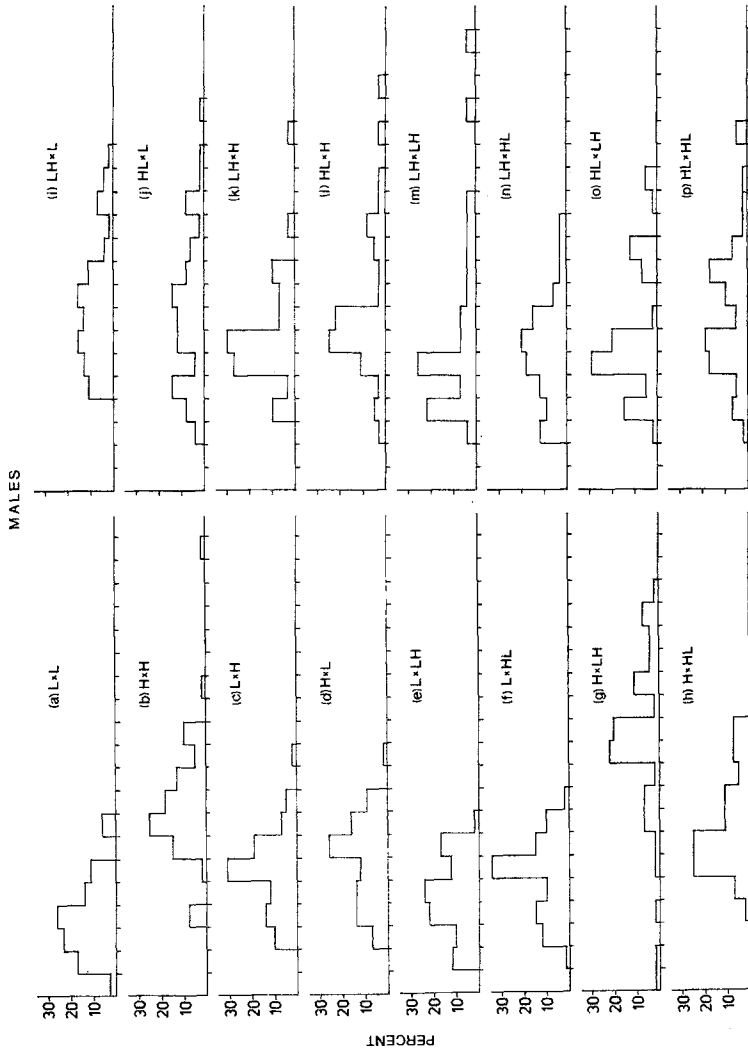

Fig. 2. The frequency distributions of the males for crosses between low and high pupal height strains of *D. melanogaster*. L. represents a low parent, H represents a high parent, and LH and HL represent an F_1 parent. (a,b) the two parental strains; (c,d) the two F_1 generations; (e–j) the eight backcrosses; (m–p) the four F_2 generations.

a finding in both female and male data sets. This pattern of pupal height inheritance was in agreement with the results of Bauer and Sokolowski (1985), who found that both the second and the third pairs of chromosomes influenced differences in pupal height.

Effect of Sex Differences on Pupal Height

ANOVA of all crosses showed a significant effect of cross ($F = 15.52$, df = 15,1342, $P < 0.001$) and sex ($F = 5.65$, df < 1,1342, $P < 0.05$) and no significant interaction between cross and sex on differences in pupal height. The sex effect reflected the finding that males had higher mean pupal heights than females in 15 of 16 crosses (Table III). Since there was a significant effect of sex on differences in pupal height, further analyses were carried out on each sex separately.

Developmental Time

Differences in developmental time were estimated by counting the number and sex of adults emerging daily from each cross. ANOVA of all crosses showed a significant effect of cross on differences in the mean number of days to emergence ($F = 5.35$, df = 15,1330, $P < 0.001$). The F_1 progeny emerged an average of 6 to 8 h earlier than the progeny of the other crosses. There was also a significant effect of sex on developmental time ($F = 19.83$, df = 1,1330, $P < 0.001$), with males tending to emerge later than females, a finding in agreement with that of Bakker and Nelissen (1963). There was no correlation between development time and pupal height for females ($r = 0.28$, df = 16) or males ($r = 0.04$, df = 16). Bakker and Nelissen (1963) found that the length of the larval period (time from egg laying to pupation) was the same in females and males of *D. melanogaster*. It was concluded therefore that the differences in pupal height found in the present study could not be accounted for by differences in development between the crosses or the sexes, in agreement with the results of Sokal *et al.* (1960).

Effects of X and Y Chromosomes on Pupal Height

To avoid the confounding effects of other factors (Table I), the effects of the X chromosomes on differences in pupal height could be analyzed only in the female backcross data. The only contrasts involving the X chromosome tested its effects in a high as compared with a low pupal height background. This contrast tested an interaction between the X chromosome and a particular background, where the background is com-

Table IV. Analyses of Variance on Log Pupal Heights for *D. melanogaster* Female Pupae[a]

Comparison	df	MS	*F*	*P* <
1. Parental and F_1 generations				
(a) Parentals	1	5.5797	99.28	0.001
(b) Reciprocal F_1	1	0.1598	2.84	ns
(c) Dominance	1	0.0299	0.53	ns
Error within vial (cross)	36	0.0562		
2. Backcrosses				
(a) Permanent cytoplasm	1	0.0428	0.42	ns
(b) Reciprocal crosses for transient maternal effects				
(i) F_1 and low parentals	1	5.1612	50.45	0.001
(ii) F_1 and high parentals	1	1.9469	19.03	0.001
(c) High versus low X chromosome in				
(i) Low background	1	2.2982	22.46	0.001
(ii) High background	1	0.1015	0.99	ns
Error within vial (cross)	69	0.1023		
3. F_2 generations				
(ii) Permanent cytoplasm	1	0.1428	0.62	ns
Error within vial (cross)	31	0.2317		

[a] For a description of comparisons see Tables I and II. ns, not significant.

prised of both chromosomal and nonchromosomal factors. The comparison showed that the effects of the X chromosomes were significant in a low autosomal and low maternal background but not a high one (Table IV).

For males effects of the Y chromosome and the interaction between Y chromosome and permanent cytoplasmic factors were not significant in the F_2 generation. In the backcrosses the difference between strains having a Y chromosome from a high versus low parental was not significant in a low background but was significant in a high background (Table V). Since the Y chromosome effect was not significant in two of three comparisons and there were no clear Y chromosome trends in these data, it was assumed that there was no consistent effect of the Y chromosome.

Maternal Effects

The ANOVAs of the data for females (Table IV) showed a highly significant maternal effect in the backcrosses. In Table III backcrosses with low parental mothers (L × LH or L × HL) showed lower mean pupal heights than their reciprocals which had an F_1 mother (LH × L or HL × L). The effect of a high parental mother (H × LH or H × HL)

Table V. Analyses of Variance on Log Pupal Heights for *D. melanogaster* Male Pupae[a]

Comparison	df	MS	*F*	*P* <
1. Parental and F_1 generations				
(a) Parentals	1	5.8751	103.98	0.001
(b) Reciprocal F_1	1	0.0633	1.12	ns
(c) Dominance	1	0.0133	0.24	ns
Error within vial (cross)	36	0.0565		
2. Backcrosses				
(a) Permanent cytoplasm	1	0.0215	0.19	ns
(b) Reciprocal crosses for transient maternal effects				
(i) F_1 and low parentals	1	4.9792	44.90	0.001
(ii) F_1 and high parentals	1	0.7263	6.55	0.050
(c) High versus low Y chromosome in				
(i) Low background	1	0.1631	1.47	ns
(ii) High background	1	1.9003	17.14	0.001
Error within vial (cross)	69	0.1109		
3. F_2 generations				
(a) Y chromosome	1	0.0074	0.03	ns
(b) Permanent cytoplasm	1	0.2957	1.36	ns
(c) Interaction between Y chromosome and permanent cytoplasm	1	0.2690	1.24	ns
Error within vial (cross)	31	0.2178		

[a] For a description of comparisons see Tables I and II. ns, not significant.

versus an F_1 mother (LH × H or HL × H) was also significant; backcrosses with high parental mothers showed higher pupal heights than their reciprocals (Table III). The maternal effect could not be attributed to permanent cytoplasmic factors as evident from both the backcross and the F_2 ANOVAs. There was no evidence for a maternal effect in the parental and F_1 data. This finding was not surprising since tests for a maternal effect using parental and F_1 crosses are confounded by differences in other factors (autosomes and sex chromosomes).

The maternal effect results found for females was mirrored in the male data (Table V). The results of the ANOVAs on the backcross data showed that the maternal effect was similar to that found in females and was not related to permanent cytoplasmic factors. The effects of permanent cytoplasmic factors were also not significant in the F_2 generation. The finding of a transient maternal effect as well as an effect of the autosomes is necessary to explain fully the pattern of inheritance of pupal height in these crosses.

DISCUSSION

We found a significant maternal effect for a late third-instar larval behavior in both the female and the male backcross data. In the Introduction we state that maternal effects on a *Drosophila* behavior may be due to permanent cytoplasmic factors, transient maternal factors, or a form of parental care. In the present study the environment external to the organism (incubator and vial conditions) was controlled and the larvae were placed into vials at a fixed density. Therefore, there was no opportunity for the maternal parents to influence offspring behavior through differences in oviposition behavior or fecundity (as in the case of Hay, 1972a,b). The methodology used in the present study also ruled out maternal effects resulting from parental age, egg retention, or egg hatchability. Since there was no parental care the maternal effect reported in this study was most likely due to differences in the internal environment of the mother, for example, differences in the components of the egg cytoplasm, which may be of a transient or permanent nature. The ANOVA results showed no effect of permanent cytoplasmic factors in the backcrosses to F_1 mothers; it did not matter whether the mother had an HL or an LH genotype. In other words, the maternal effect was not transmitted "intact" from generation to generation; it did not matter which grandmother was used. We conclude that this maternal effect on pupal height follows the pattern of inheritance of a transient maternal effect. The phenotype of the offspring was more like the phenotype of the mother than expected from a purely additive autosomal model of inheritance.

Further evidence of the presumptive importance of maternal factors is found in the significant effect of the X chromosome in a low pupal height background. This significant effect can represent epistatic interactions between the X chromosomes and the low autosomal background and/or between the X chromosome and the low maternal background. In light of the highly significant transient maternal effect it is reasonable to speculate that the significant effect of the X chromosome in the low pupal height background probably reflects an interaction between the X chromosomes and the maternal effect. This hypothesis could be tested by studying the effect of the X chromosome in different maternal backgrounds when autosomal factors are held constant.

The F_1 phenotypes had intermediate pupal heights and did not show a maternal influence on the behavior (the F_1 generations were not significantly different from each other). The pattern of inheritance found in the present study is paralleled by the results of Le Pape and Lassalle (1981) in their study on swimming speed in inbred mice. They also found

transient maternal effects expressed in the backcross but not the F_1 generations. They state that their results may be related to the relative levels of inbreeding in the F_1 backcross and generations. They suggested that mice (and *Drosophila*) in the F_1 generation are highly heterozygous and may be buffered against environmental effects (such as maternal effects) and that backcrosses which are homozygous at 50% of their loci may be more sensitive to differences in the maternal environment. The methodology used by Le Pape and Lassalle (1981) could not distinguish among the effects of the egg cytoplasm, intrauterine environment, and parental care. In the present study the potential effect of parental care (oviposition site choice, fecundity, egg hatchability) was eliminated experimentally. To distinguish among these three factors in mice, cross-fostering and ovarian transplantations are required (Wahlsten, 1979). Wakasugi (1973) performed ovarian transplants in mice and found that a maternal effect on female fertility was due to defects in the eggs rather than differences in the uterine environment.

Drosophila spend most of their larval life foraging for food. Midway through the third larval instar there is a switch in behavior from foraging to wandering (Sokolowski *et al.*, 1984). Wandering larvae leave the feeding substrate in search of a pupation site. Wandering larvae treat food (yeast) and nonfood (agar) substrates similarly. This switch in behavior is probably mediated by changes in physiology. Strain differences in larval behavior in the foraging stage do not show maternal effects in their pattern of inheritance, for example, larval foraging path length (Bauer and Sokolowski, 1984; de Belle and Sokolowski, 1987). The maternal effect reported in the present study is expressed only in the wandering stage. It is this fact that may give us a clue from a mechanistic viewpoint as to what aspects of the physiology of wandering behavior or the morphological structures employed in wandering may be involved in this maternal effect. Differences in morphology (for example, segmentation pattern) may influence larval locomotor behavior on dry substrates encountered during wandering behavior. The possible effects of maternal gene products (mRNA's or proteins) on cuticular segmentation and differention have been reported by Garcia-Bellido and Del Prado (1979).

In the present study, the parental strains differed in the origin of both the second and the third pairs of chromosomes. Both of these chromosomes carry genes which influence pupal height (Bauer and Skolowski, 1985). In the future, we plan to determine whether and in what manner the maternal effect interacts with the genotype of the offspring. This could readily be determined by performing crosses between the strains described by Bauer and Sokolowski (1985) which differ in only one pair of chromosomes. If B1B1 and B15B1 (which have identical third chromo-

somes) are used as the parental strains, the interaction between the second pair of chromosomes and the maternal effect could be examined.

It is also of interest to speculate as to the conditions under which a transient maternal effect on pupation behavior could evolve. In a stable environment, a successful strategy would be for a larva to bias its pupation behavior in the direction of its mother's pupation behavior since by definition the mother's survivorship to oviposition indicates that her pupation behavior was successful. However, when environmental fluctuations are unpredictable it is not to a larva's advantage to pupate like its mother. One example of selection acting on pupation behavior is the relationship between the water content of the soil and pupal survivorship. In dry soils, larvae that pupate in or near the fruit have greater pupal survivorship than those pupating on the soil. In relatively wetter soils, the converse is true (Sokolowski, 1985). One possible prediction from this conceptual model is that the size of the maternal influence on pupation behavior should be larger in stable environments and smaller or nonexistent in environments which fluctuate in an unpredictable fashion.

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