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Interaction between the effects of maternal and larval levels of nutrition on pre-adult survival in *Drosophila melanogaster*

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ABSTRACT

Drosophila melanogaster is used extensively for studies of life-history trade-offs involving acquisition and allocation of nutritional resources. Yet we know very little about the effects of level of parental nutrition on offspring fitness traits, and whether parental and offspring nutrition interact in their effect on offspring phenotype, although such interactions are known in other organisms. We looked for such parental effects and interactions by assaying pre-adult survivorship and dry weight at eclosion on individuals from a long-standing laboratory population of *D. melanogaster* subjected to four combinations of maternal (rich or poor food) and offspring (rich or poor food) nutritional environments. We saw no effect of maternal food level on offspring dry weight at eclosion. On average, females were heavier than males, and flies given rich food as larvae were heavier than those raised on poor food as larvae. Levels of maternal and offspring food interacted significantly in their effect on pre-adult survivorship, with the highest survivorship being seen in individuals from the combination of poor maternal and rich offspring food; survivorship did not differ significantly among the other three treatments. We suspect that some aspect of egg provisioning by mothers reared on poor food may be involved in this interaction, as we found that females reared on poor food laid eggs that were 28% heavier than those from females reared on rich food. This difference, however, was not significant. Although the underlying basis for the interaction between levels of maternal and offspring food in their effect on pre-adult survivorship is not clear at this time, we believe that the observation of such an interaction suggests caution in interpreting results from dietary manipulation experiments in *Drosophila*, in which maternal nutrition is typically not treated as a factor in the experimental design.

Keywords: *Drosophila*, dry weight, fitness, larval survivorship, life history, maternal effect, nutrition.

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INTRODUCTION

The possible adaptive significance of non-genetic parental effects has attracted interest recently (Mousseau and Fox, 1998; Bateson and Martin, 1999). In particular, studies across a range of taxa suggest that the nutritional status of parents and offspring can have major effects on fitness-related traits in the offspring (Shibata and Rollo, 1988; Nelson, 1991; Glazier, 1992; Gliwicz and Guisande, 1992; Rossiter, 1993; Schmid and Dolt, 1994; Lin and Dunson, 1995; Fox *et al.*, 1997). Even more interesting is the growing number of examples of how parental and offspring nutritional environments can interact to affect the phenotypic expression of parental effects in the offspring (reviewed by Rossiter, 1998). In mammals, including humans, maternal nutritional status can interact with subsequent offspring nutritional status, such that offspring fitness is reduced when maternal and offspring nutritional status are negatively correlated (Dahri *et al.*, 1991; Iglesias-Barreira *et al.*, 1996; Ravelli *et al.*, 1998). For example, the thrifty phenotype hypothesis suggests that poor maternal nutrition predisposes the offspring towards an alternative developmental pathway that will prove beneficial if the offspring also experience a poor nutritional environment, but can be harmful if the offspring experience a nutrition-rich environment (Hales and Barker, 1992; Bateson and Martin, 1999).

In addition to being of interest in their own right as evolved adaptations that may help organisms cope with varying environments (Lacey, 1998), parental effects can potentially be a major confounding factor in experiments in organismal biology, especially when the parental and offspring environments interact in their effect on the offspring phenotype (Crill *et al.*, 1996). Parental effects and interactions involving levels of nutrition are of particular relevance to experimental studies of life-history evolution, because much attention in such studies is focused on trade-offs surrounding the acquisition and allocation of nutritional resources (Van Noordwijk and De Jong, 1986; Partridge and Sibly, 1991). If the phenotypic effects of level of offspring nutrition can be qualitatively altered by level of parental nutrition, there is clearly reason for concern, as the experimental design for such studies typically does not include level of parental nutrition as a factor (Partridge *et al.*, 1987; Leroi *et al.*, 1994; Chippindale *et al.*, 1997; Borash and Ho, 2001).

The fruit fly *Drosophila melanogaster* has been a favoured model system for experimental studies of nutrition-related life-history trade-offs (Trevitt *et al.*, 1988; Hillesheim and Stearns, 1992; Joshi and Mueller, 1996; Chippindale *et al.*, 1998; Djawdan *et al.*, 1998; Harshman *et al.*, 1999) and, in the context of laboratory selection experiments, is increasingly being used for comparative physiology as well (Gibbs, 1999). Yet very little is known about the possible interaction between parental and offspring environments in determining offspring phenotype in *D. melanogaster*, especially in terms of nutritional effects. Effects of parental age (Butz and Hayden, 1961) and rearing temperature (Crill *et al.*, 1996; Watson and Hoffmann, 1996) on offspring fitness components have been observed in *D. melanogaster*, but nutritional level effects, to our knowledge, have not been studied. However, in species other than *Drosophila*, there is evidence of an interaction between the effects of parental and offspring nutritional environments on offspring phenotypes (Glazier, 1992; Parichy and Kaplan, 1992; Brett, 1993; Lin and Dunson, 1995; Ravelli *et al.*, 1998). In this paper, we report results from an experiment on *D. melanogaster* in which we sought answers to the following two questions: (1) Does maternal nutritional environment affect egg weight? (2) Do maternal and offspring nutritional environments interact in their effect on offspring survivorship from egg to eclosion, and on offspring dry weight at eclosion?

MATERIALS AND METHODS

Experimental flies

We used a large, long-term laboratory-reared population of *D. melanogaster* (JB-1), which is one of a set of four replicate populations maintained in our laboratory, whose ancestry and maintenance have been described previously in detail (Sheeba *et al.*, 1998). The population has been maintained in incubators in the laboratory at 25°C, about 90% relative humidity and constant light, on a 21 day discrete generation cycle for about 200 generations on banana-jaggery food (see Table 1 for details of foods used in this study). The number of breeding adults is about 1800 and the adults are maintained in a Plexiglas cage (25 × 20 × 15 cm) with abundant food topped with a generous smear of yeast-acetic acid paste. Eggs are collected from these flies by placing a fresh petri plate with food into the cage for 18 h. The eggs collected off the food plate are then dispensed into 40 vials (9 cm in height and 2.4 cm in diameter) at a density of 60–80 eggs per vial; each vial contains about 6 ml of food. On the 18th day after egg collection, all the eclosed flies are collected into the cage and, 3 days later, eggs are collected to start the next generation.

For this study, we collected eggs from the running culture and dispensed them into vials containing 6 ml of either rich or poor food (see Table 1) at an exact density of 60 eggs per vial. We set up 120 vials each of poor and rich food in this manner. On the 18th day after egg laying, we collected all eclosed adults into cages and supplied them with the same type of food (rich or poor) that they experienced as larvae. The females in these cages are the 'mothers' for the assays described below, and rich or poor maternal food thus refers to the food on which the mother was reared, both as a larva and as an adult. We collected eggs for the assays by placing petri plates with regular food in the cages for a 4 h egg-laying window.

Egg weight assay

We collected a total of 480 eggs laid by females reared on poor or rich food from the food plates placed in the respective cages, rinsed them in water to remove any adhering food medium, dried them on filter paper, and then weighed them in eight batches of 30 eggs each per maternal nutritional level.

Table 1. The composition of 1 litre each of regular food (used for maintenance of the running culture of the population used in the study) and the rich and poor food used for the assays

Ingredient	Regular food	Rich food	Poor food
Banana (g)	205	205	410
Barley flour (g)	25	25	50
Jaggery (unrefined cane sugar) (g)	35	35	none
Yeast (g)	36	72	none
Agar (g)	12.4	12.4	12.4
Ethanol (ml)	45	45	45
Water (ml)	180	180	90
p-Hydroxymethylbenzoate (g)	2.4	2.4	2.4

Pre-adult survivorship assay

We collected eggs laid by females reared on poor or rich food from the food plates placed in the respective cages, and dispensed them into vials containing 6 ml of either rich or poor food at a density of 60 eggs per vial. We set up about 20 such vials for each maternal food × larval food combination and recorded the proportion of eggs surviving to become pupae and adults in each vial.

Adult dry weight assay

We collected freshly eclosed flies from the vials used for the pre-adult survivorship assay, killed them by freezing, sorted them by sex, dried them for 36 h at 70°C, and then weighed them in batches of five males or five females. We weighed eight such batches for each maternal food × larval food × sex combination.

Statistical analysis

We implemented all analyses on STATISTICA™ for Windows Release 5.0 B (StatSoft, 1995). We subjected the egg weight data to a one-way analysis of variance (ANOVA) with maternal food as the sole fixed factor. We transformed the larval survivorship, pupal survivorship and egg-to-adult survivorship data by an arcsin square root transformation (Freeman and Tukey, 1950), and then subjected them to a two-way ANOVA with maternal food and larval food as the two fixed factors. Data on dry weights at eclosion were subjected to three-way ANOVA with maternal food, larval food and sex treated as fixed factors. We used Tukey's HSD test for all pairwise multiple comparisons.

RESULTS

The mean (± standard error) weight per egg of eggs laid by mothers reared on poor food (0.0113 ± 0.0021 mg) was about 28% greater than that of eggs laid by mothers reared on rich food (0.0088 ± 0.0018 mg), but the difference was not significant ($F_{1,14} = 0.83$, $P = 0.38$). Mean larval survivorship (fraction surviving from egg to pupation) was about 0.8 in three of the four combinations of maternal and larval food levels, and was significantly greater ($P < 0.05$) in the case of larvae growing on rich food when their mothers had been reared on poor food (Fig. 1). Pupal survivorship was high and did not differ among combinations of maternal and larval food levels (Fig. 1). Egg-to-adult survivorship showed the same pattern as larval survivorship, and the patterns observed in Fig. 1 are supported by the ANOVA results, with both larval and egg-to-adult survivorship showing significant effects of larval food, and the maternal food × larval food interaction (Table 2).

The only significant effects on dry weight at eclosion were those of larval food, sex, and the larval food × sex interaction (Table 3). Females were significantly heavier than males, and individuals reared as larvae on rich food were significantly heavier than those reared as larvae on poor food (Fig. 2). The significant larval food × sex interaction is due to the fact that the dry weight of females increased proportionately more than that of males (by 72% as opposed to 66%) when reared as larvae on rich rather than poor food (Fig. 2).

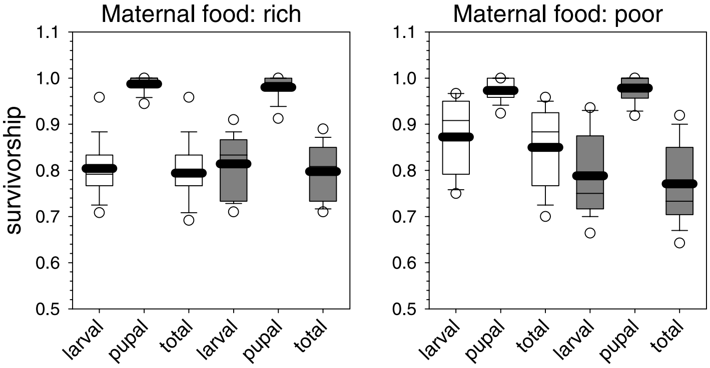

Fig. 1. Box and whisker plot of survivorship in different pre-adult life stages under various combinations of maternal and larval nutrition. Boxes indicate the 25th and 75th percentiles and median, whereas the whiskers indicate the 10th and 90th percentiles. Circles indicate the 5th and 95th percentiles, and the mean is indicated by a thick line. White and grey boxes represent the treatments with rich and poor larval food, respectively.

Table 2. Summary of results of three separate analyses of variance carried out on arcsin square root transformed survivorship in different pre-adult life stages under various combinations of maternal and larval nutrition

	Larval		Pupal		Egg-to-adult	
Effect	$F_{1,85}$	P	$F_{1,85}$	P	$F_{1,85}$	P
Maternal food	2.42	0.12	1.76	0.19	1.23	0.27
Larval food	5.15	0.03	0.11	0.74	5.48	0.02
Maternal food × larval food	7.42	0.01	2.14	0.15	5.43	0.02

Table 3. Results of an analysis of variance on dry weight at eclosion (grams per 5 flies) of individuals emerging from various combinations of maternal and larval nutrition

Effect	d.f.	MS	F	P
Maternal food	1	1.9×10^{-37}	1.4×10^{-29}	0.99
Larval food	1	7.4×10^{-6}	545.36	<0.001
Sex	1	2.6×10^{-6}	188.01	<0.001
Maternal food × larval food	1	1.9×10^{-37}	1.4×10^{-29}	0.99
Maternal food × sex	1	5.6×10^{-9}	0.4131	0.52
Larval food × sex	1	2.5×10^{-7}	18.361	<0.001
Maternal food × larval food × sex	1	6.3×10^{-10}	0.0459	0.83
Error	56	1.4×10^{-8}		

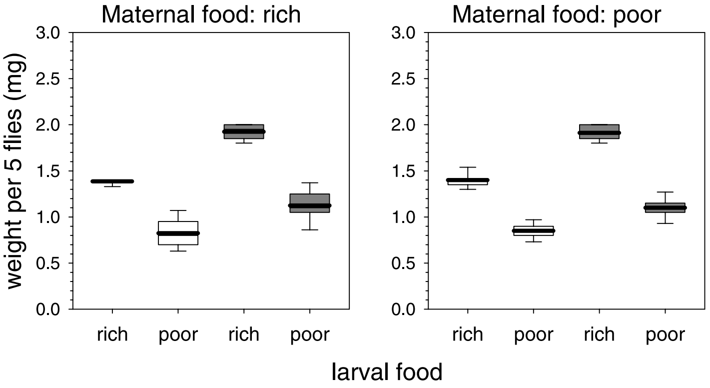

Fig. 2. Box and whisker plot of dry weight at eclosion under various combinations of maternal and larval nutrition. Boxes indicate the 25th and 75th percentiles and median, whereas the whiskers indicate the 10th and 90th percentiles. Circles indicate the 5th and 95th percentiles, and the mean is indicated by a thick line. White and grey boxes represent males and females, respectively.

DISCUSSION

Our results provide clear evidence for an interaction between levels of maternal and larval nutrition on larval and, therefore, egg-to-adult survivorship (Fig. 1, Table 1). Larvae whose mothers were reared on poor food and who themselves were reared on rich food had higher survivorship than larvae from all other combinations of maternal and larval food. We do not presently know the reason for this higher survivorship, although it is possible that some aspect(s) of egg provisioning by mothers experiencing poor food enhances the ability of the larvae hatching from those eggs to do better when they get rich food. In our experiment, mothers reared on poor food did lay heavier eggs than those reared on rich food, but the difference was not statistically significant. Lin and Dunson (1995) observed a similar interaction between levels of parental and offspring nutrition on offspring fitness components in the estuarine fish *Rivulus marmoratus*. In their study, larvae from parents that experienced poor nutrition were able to mature earlier and were heavier when reared in a high nutrition environment, compared with larvae from parents that experienced good nutrition. Unfortunately, the physiological mechanism for this interaction was unclear (Lin and Dunson, 1995).

We observed no significant effect or interaction of maternal nutrition on the dry weight of offspring at eclosion (Fig. 2, Table 2), suggesting that whatever effect is responsible for larvae from mothers reared on poor food surviving better on rich food does not yield a greater efficiency of assimilation. It is, however, possible that offspring of mothers reared on poor food may be superior in terms of lipid storage than individuals from other combinations of maternal and larval nutrition. Individuals from *D. melanogaster* populations adapted to very high larval densities are not heavier at eclosion than those from ancestral control populations, but do have a greater lipid content, which can have an effect on subsequent fecundity and stress resistance (Borash and Ho, 2001).

The results from our experiment are clearly inconsistent with the thrifty phenotype hypothesis (Hales and Barker, 1992; Bateson and Martin, 1999), which would require treatments with a positive correlation between levels of maternal and offspring food to result in superior offspring fitness, compared with treatments in which levels of maternal and offspring food are negatively correlated. However, given the diversity of the various patterns of interactions between parental and offspring environments in affecting offspring phenotype (Rossiter, 1998), it is unlikely that generalizations about possible adaptive patterns of parental effects and interactions would have broad validity across taxa. It is far more likely that the kinds of parental effects and interactions we see in any taxon will depend in a complex way on the ecology, physiology and evolutionary history of the population being studied.

Overall, our results clearly show that at least one important component of fitness in *D. melanogaster* is affected by levels of maternal and offspring nutrition in a complex manner. For example, the increased survivorship of larvae on rich food would not have been detected in an experiment in which mothers were raised on rich food, which is typically the case in laboratory experiments on *Drosophila*. Our results, therefore, highlight the need for caution in interpreting results from nutritional manipulation experiments in *Drosophila*, and suggest that a more detailed investigation of maternal nutrition effects on fitness-related traits of *Drosophila* individuals subjected to varying nutritional regimes is necessary.

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