Effects of parental larval diet on egg size and offspring traits in *Drosophila*

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Abstract

If mother's nutritional status predicts the nutritional environment of the offspring, it would be adaptive for mothers experiencing nutritional stress to prime their offspring for a better tolerance to poor nutrition. We report that in *Drosophila melanogaster*, parents raised on poor larval food laid 3-6% heavier eggs than parents raised on standard food, despite being 30% smaller. Their offspring developed 14 h (4%) faster on the poor food than offspring of well-fed parents. However, they were slightly smaller as adults. Thus, the effects of parental diet on offspring performance under malnutrition apparently involve both adaptive plasticity and maladaptive effects of parental stress.

Key words: maternal effects, parental effects, egg size, nutritional stress, plasticity, *Drosophila*

Introduction

Parental genotype and environment often influence offspring fitness through non-genetically transmitted parental effects. Such effects may be maladaptive, e.g., malnourished parents may produce offspring of poorer quality (parental stress hypothesis). However, parents may also respond to environmental cues in ways that enhance offspring fitness. In particular, if the nutritional conditions experienced by the mother and offspring are positively correlated, mothers subject to nutritional stress would be favored to induce plastic changes in the offspring that make the latter more tolerant to nutritional stress. This adaptive hypothesis thus predicts that fitness of offspring on poor diet would be enhanced if their parents also experienced poor diet (Badyaev and Uller 2009; Mousseau and Fox 1998).

One potential mechanism of such adaptive parental effects involves adjustment of investment per offspring, which in organisms lacking parental care can be approximated by egg or newborn size (Azevedo et al. 1997; Mousseau and Fox 1998). Life history theory predicts that under adverse conditions the optimal trade-off between offspring size and number is expected to shift towards fewer but better provisioned offspring (Roff 1992; Smith and Fretwell 1974). Natural selection should thus favor mothers that invest more in individual offspring in response to cues indicative that offspring would experience nutritional stress. One such cue would be the mother's own nutritional environment.

The prevalence of such adaptive parental effects remains unclear. An increase in egg or newborn size in response to poor parental nutrition or high competition has been reported, e.g., in seed beetles (Kawecki 1995), cockroaches (Barrett et al. 2009), *Daphnia* (McKee and Ebert 1996), and bryozoans (Allen et al. 2008), but more often a decrease or no effect was observed (reviewed by Fox and Czesak 2000). Beneficial effects of poor parental nutrition on offspring themselves facing nutritional stress have been observed, e.g., in *Daphnia* (Gliwicz and Guisande 1992), butterflies (Rotem et al. 2003) and mosquitoes (Grech et al. 2007), similar plasticity has been suggested in humans (Hales and Barker 2001). However, there are numerous examples of adverse effects of even mild parental nutritional stress (e.g., Bonduriansky and Head 2007; Diss et al. 1996; Jones and Widemo 2005; Kyneb and Toft 2006).

Here we study the effects of parental larval nutrition (poor versus standard) in *Drosophila melanogaster*. First, we test the adaptive hypothesis that females raised on poor diet should produce larger eggs, despite having a smaller body size. Second, we study the effect of parental nutrition on offspring fitness traits (egg-to-adult viability, developmental time and adult body size). A simple stress hypothesis would predict that parents raised on poor diet would produce offspring of low quality, which survive poorly, take longer to develop, and reach a smaller adult size than offspring of well-fed parents. These differences would be particularly manifest if the offspring themselves also developed under nutritional stress. In contrast, according to the adaptive maternal effects hypothesis, mothers raised on poor food would "prime" their offspring for development under nutritional stress such that when the offspring themselves are raised on poor food, they perform better than the offspring of mother raised on a richer diet.

In the only *Drosophila* study that addressed these questions, mothers maintained on a poor food tended to lay larger eggs, but the difference was not significant. Furthermore, poorly fed mothers produced offspring that survived better to adulthood on a rich food, compared to offspring of well-fed mothers, with no difference on the poor food (Prasad et al. 2003). This pattern is not predicted by either the stress hypothesis or the adaptive maternal effects hypothesis. However, the media used in that study differed in the type as well as the concentration of nutrients, with the poor food containing a greater amount of starch than the rich food. In our study, food quality was reduced by diluting the standard food recipe. In contrast to Prasad et al. (2003) we only manipulated the parental larval diet; all parents were maintained as adults on standard food. This may be more ecologically relevant; in nature *Drosophila* adults tend to use a greater variety of food sources than larvae (Shorrocks 1975).

Materials and methods

We used an inbred laboratory strain Canton-S (additional data on two strains recently derived from nature are reported in the Electronic Supplementary Material). The stock had been maintained for several years in our laboratory on a cornmeal medium (30g sucrose, 60g glucose, 12.5g dry yeast, 50g cornmeal, 0.5g $MgSO_4$, 0.5g $CaCl_2$, 30ml ethanol, 6ml propionic acid, and 1g nipagin per liter; henceforth referred as standard food).

Flies for the parental generation were raised at the density of 100 eggs per vial, at 25ºC with 30ml of either the standard food, or on poor food containing ¼ of the amounts of sugars, yeast and cornmeal relative to the standard food. The assays were carried in two separate experiments. In experiment 1, vials with parents raised on poor food were initiated four days earlier than those on standard food, to compensate for the development on poor food taking four days longer. The eggs for measurement of eggs size and offspring traits were collected when parents on both food types were 4-6 days old counting from eclosion. In experiment 2,

parental generation on both food types was established simultaneously and eggs were collected 20 days later, when the parents raised on the poor food were 4-6 days old and those raised on standard food were 8-10 days old. In both experiments the parents were transferred to new vials with standard food and live yeast two days before egg collection, which occurred in mass oviposition.

For egg-weight, five batches of 30 eggs (from parents raised in five different vials) per experiment × maternal diet combination were rinsed with water, dried on filter paper and weighed to the nearest microgram. To assay offspring traits, four vials with standard food and four with poor food (arranged in two blocks obtained from parents raised in two different vials and offset by several days) were set up for each experiment × parental diet combination, each seeded with 100 eggs. The number of larvae pupating in each vial was scored every 24 hours to estimate the time to pupation and pupation success. Adults eclosing daily in each vial were counted and collected (to obtain egg-to-adult viability and developmental time). Twelve females were randomly chosen from the day of peak emergence in each vial, dried at 70ºC for 3 days and weighed individually.

To simplify the analysis, we first calculated the mean egg weight per batch and the means of offspring traits per vial. Viability (the proportion of eggs that developed into adults) was angularly transformed. The pupal period was estimated as the difference between the egg-to-adult developmental time and the time to pupation. These values were analyzed with ANOVA using JMP statistical software. Parental diet, offspring diet and experiment were fixed factors; block was included as a random factor nested within experiment. For offspring traits, we also carried out separate analysis for the two levels of offspring diet. Interactions and block effect with $P > 0.2$ were excluded from the final models.

Results

Females raised on poor food laid heavier eggs than mothers raised on standard food ($F_{1,16} = 10.58$, $P = 0.005$; Fig.1). This effect was consistent between experiments (interaction $F_{1,16} = 0.66$, $P = 0.43$); the overall greater weight of eggs in experiment 2 ($F_{1,16} = 22.4$, $P = 0.0002$) is likely due to some difference in egg handling (the order of weighing was randomized within experiments).

In both experiments offspring raised on poor food pupated earlier and showed a slightly smaller adult weight if their parents also developed on poor food (Fig.2, Table.1). No effect of parental diet was detected if the offspring were raised on the standard food, although the parental × offspring diet interaction was only marginally significant for time to pupation. In contrast, poor parental diet improved viability on standard but not on poor food, particularly in experiment 2 (Fig.2, Table.1). The length of the pupal period was unaffected by parental diet (Fig.2, Table.1).

Discussion

Females raised on poor food weigh 30% less than those raised on the standard food (see also Kolss et al. 2009). Given that intraspecific correlations between maternal size and egg size in arthropods are typically positive (Azevedo et al. 1997), one would expect their eggs also to be smaller. Yet, in both experiments females raised on the poor food laid 3-6% heavier eggs, confirming the trend observed by Prasad et al. (2003) and indicating a specific, evolved plastic response. The same response was also observed in two outbred strains recently derived from natural populations (Electronic Supplementary Material). Presumably, the larger egg size reflects enhanced egg provisioning, consistent with the adaptive response predicted by life history theory (Smith and Fretwell 1974).

The prediction of adaptive parental effects hypothesis was only upheld for one offspring trait, time from oviposition to pupation on poor food: parents raised on poor food produced offspring which pupated on average about 14h (4%) earlier than offspring of parents raised on standard food. No such effect was detected when the offspring developed on standard food; this (marginally significant) interaction between maternal and offspring diet is also consistent with the adaptive parental effects hypothesis. Development on poor food is generally slow, and under these conditions being able to develop faster may be particularly advantageous. First, the already initially poor nutritional environment will deteriorate as the meager resources are used up by competing larvae and waste products accumulate. Second, even in the absence of competition the larval food sources (decomposing fruit) are likely to become increasingly unsuitable due to rotting or desiccation.

Other offspring traits did not conform to the adaptive parental effects hypothesis. The slightly smaller body weight of daughters whose parents were raised on poor food possibly reflects a trade-off with the faster development of these offspring, but is also consistent with the maternal stress hypothesis. Poor parental food enhanced viability of Canton-S flies on standard rather than poor food, similar to a result reported by Prasad et al. (2003). However, additional data from two other strains indicate that this viability effect may be strain-specific, in contrast to the effect on time to pupation and body weight, which were consistent among strains (Electronic Supplementary Material).

While in principle a non-genetic paternal effect on developmental time or body weight cannot be excluded, such paternal effects have been rarely observed in *Drosophila* (Pitnick and Karr 1998). Thus, the effects of parental diet on developmental time and weight of offspring raised on poor food are presumably mediated by maternal effects. We cannot say to what extent the faster development of offspring of parents raised on poor food is due to the larger egg size rather than to maternal effects mediated otherwise. On the one hand, larger egg size in insects, including *Drosophila*, is typically associated with shorter development (Azevedo *et al.* 1997). On the other hand, egg size also usually correlates positively with larval viability and adult size (Azevedo *et al.* 1997); if anything, offspring of mothers raised on the poor food showed opposite trends.

One can speculate that without the plastic increase in eggs size, the reduction in offspring viability and body size would be even greater. If so, both the parental stress hypothesis and the adaptive parental effects hypothesis may be true. A parental history of malnourishment may have an adverse effect on some aspect of offspring performance, but adaptive plastic responses may act to alleviate these adverse effects. For some traits (here time to pupation) the plastic response would be strong enough for the offspring of the malnourished parents to perform better under nutritional stress than the offspring of well-fed parents. This would require that the effects of parental diet on offspring performance are mediated by multiple underlying variables rather than just egg size, which is also what the results of this study suggest.

Acknowledgements

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Smith, C.C. & Fretwell, S.D. 1974. Optimal balance between size and number of offspring. *Am Nat* 108, 499-506.
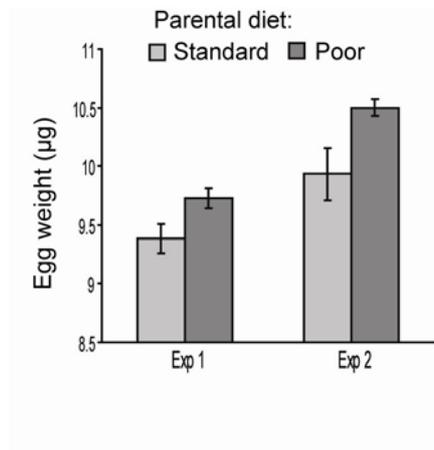
Figures

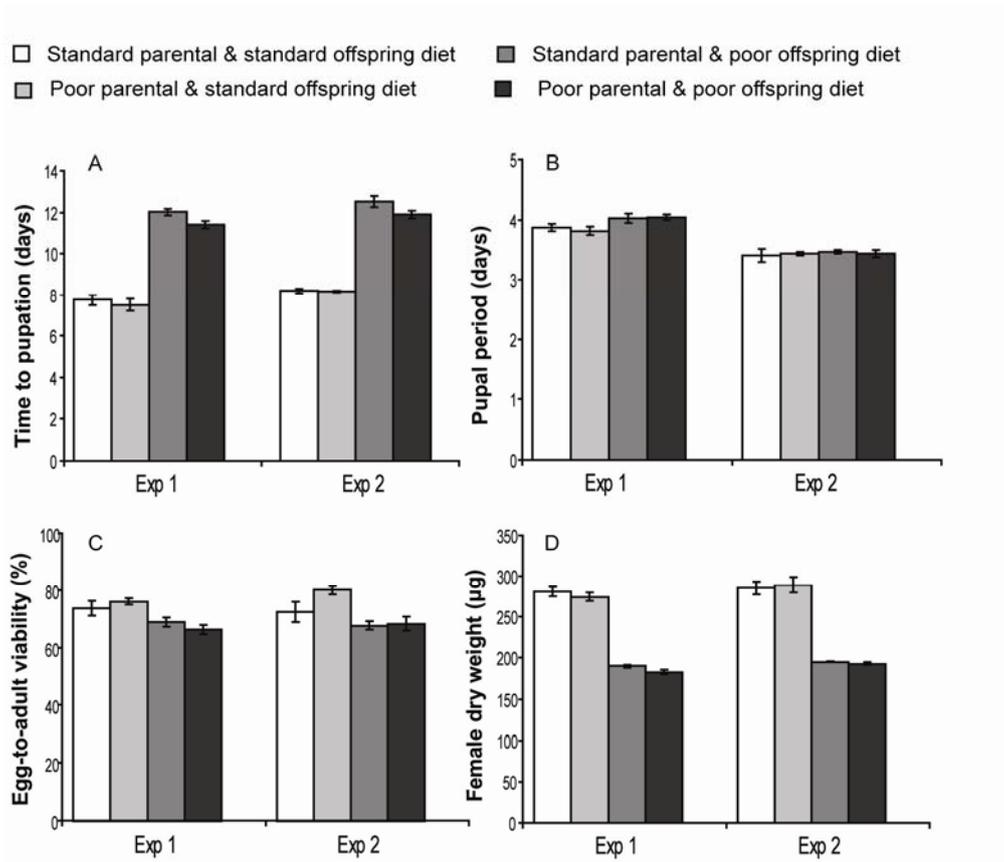Figure 1: Egg weight (mean ± S.E) as a function of maternal larval diet.

Figure 2: Offspring traits (means ± S.E) as a function of parental and offspring diets: (A) time from oviposition to pupation, (B) the duration of the pupal stage, (C) egg-to-adult viability and (D) female dry weight.

Table 1: Summary of results of analyses of variance (F statistics and their significance) on the offspring traits, analyzed jointly on both offspring diets (A), and separately for each level of offspring diet (B). Interactions indicated with "−" had *P* >0.2 and were excluded from the model.

A	Both offspring diets						
	Offspring diet	Parental diet	Exp	Block	Offspring × Parental diet	Offspring diet × Exp	Parental diet × Exp
Time to pupation	$F_{1,25}=1020.6$***	$F_{1,25}=8.8$***	$F_{1,25}=7.0$	$F_{2,25}=2.5$	$F_{1,25}=3.5$†	−	−
Pupal period	$F_{1,25}=7.0$†	$F_{1,25}=0.1$	$F_{1,25}=39.7$*	$F_{2,25}=3.6$*	−	$F_{1,25}=3.8$†	−
Egg-adult viability	$F_{1,24}=33.4$***	$F_{1,24}=2.5$	$F_{1,24}=0.2$	$F_{2,24}=3.0$†	$F_{1,24}=4.7$*	−	$F_{1,24}=2.7$
Female dry weight	$F_{1,28}=665.5$***	$F_{1,28}=0.6$	$F_{1,28}=5.8$*	−	−	−	−

B	Standard offspring food				Poor offspring food			
	Parental diet	Exp	Block	Parental diet × Exp	Parental diet	Exp	Block	Parental diet × Exp
Time to pupation	$F_{1,13}=0.5$	$F_{1,13}=8.5$*	−	−	$F_{1,11}=33.2$***	$F_{1,11}=1.4$	$F_{2,11}=15.7$***	−
Pupal period	$F_{1,11}=0.04$	$F_{1,11}=21.8$*	$F_{2,11}=1.6$	−	$F_{1,11}=0.01$	$F_{1,11}=31.6$*	$F_{2,11}=5.7$*	−
Egg-adult viability	$F_{1,10}=8.0$*	$F_{1,10}=0.1$	$F_{2,10}=6.6$*	$F_{1,10}=2.6$	$F_{1,13}=0.2$	$F_{1,13}=0.1$	−	−
Female dry weight	$F_{1,11}=0.01$	$F_{1,11}=1.6$	$F_{2,11}=1.2$	−	$F_{1,13}=6.5$*	$F_{1,13}=15.9$**	−	−
Exp:- Experiment								
†P < 0.08, *P < 0.05, **P < 0.01, ***P < 0.001; all remaining P > 0.1								

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Electronic Supplementary Material

Egg size and offspring traits in two additional fly strains

Two outbred populations of *D. melanogaster* derived from females caught around two sites in Switzerland, Valais (2007) and Fribourg (2008) were assayed for effects of parental nutrition on egg size and offspring traits, as in experiment 2 reported in the main paper. As in the CantonS strain reported in the main paper, in both outbred strains mothers bred on poor food laid larger eggs (Figure S1). Also, parental diet affected the time to pupation and adult weight of offspring raised on poor food similarly as in the CantonS strain, confirming the conclusions of the main paper (Figure S2, Table S1). However, in contrast to the CantonS results presented in the main paper, poor parental diet significantly reduced offspring viability in both outbred strains, irrespectively of offspring diet (Figure S2, Table S1). Thus, the effects of parental diet on offspring viability seem to be strain-dependent.
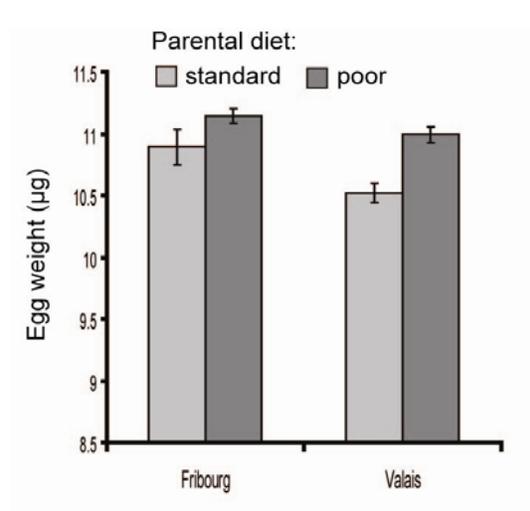

Figure S1: Weight (mean ± S.E) as a function of maternal larval diet and strain. The egg size varied significantly between the strains ($F_{1,17}$=7.66, p=0.0132) and poor food mothers laid heavier eggs than mothers raised on standard food ($F_{1,17}$=14.58, p=0.0014).
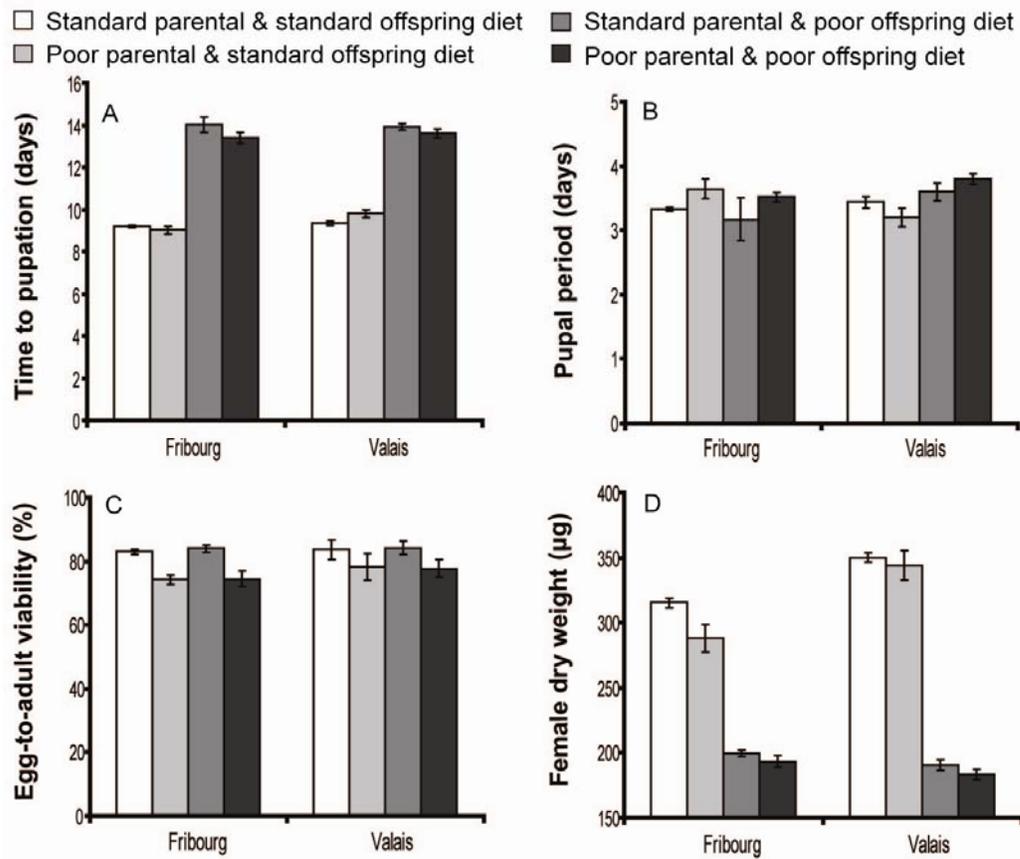
Figure S2: Offspring traits (means ± S.E) as a function of parental and offspring diets in two strains, Fribourg and Valais: (A) time from oviposition to pupation, (B) the duration of the pupal stage, (C) egg-to-adult viability and (D) female dry weight.

Table 1: Summary of results of analyses of variance (F statistics and their significance) on the offspring traits, analyzed jointly on both offspring diets (A), and separately for each level of offspring diet (B). Interactions indicated with "−" had $P > 0.2$ and were excluded from the model.

A	Both offspring food types					
	Parental diet	Offspring diet	Strain	Parental x Offspring diet	Offspring diet x Strain	Parental diet x Strain
Time to pupation	$F_{1,1}=0.49$	$F_{1,1}=459.88*$	$F_{1,1}=0.92$	$F_{1,25}=4.62*$	$F_{1,25}=2.09$	$F_{1,25}=2.57$
Pupal period	$F_{1,1}=0.76$	$F_{1,1}=0.21$	$F_{1,1}=0.09$	−	$F_{1,26}=5.59*$	$F_{1,26}=2.49$
Egg-adult viability	$F_{1,28}=18.52***$	$F_{1,28}=0.02$	$F_{1,28}=1.7$	−	−	−
Female weight	$F_{1,26}=6.76*$	$F_{1,1}=23.07$	$F_{1,1}=0.42$	−	$F_{1,26}=37.98***$	−

B	Standard offspring food			Poor offspring diet		
	Parental diet	Strain	Parental diet x Strain	Parental diet	Strain	Parental diet x Strain
Time to pupation	$F_{1,1}=0.22$	$F_{1,1}=2.25$	$F_{1,12}=5.08*$	$F_{1,12}=4.22†$	$F_{1,12}=0.06$	−
Pupal period	$F_{1,1}=0.02$	$F_{1,1}=0.38$	$F_{1,12}=5.68*$	$F_{1,13}=2.16$	$F_{1,13}=3.72†$	−
Egg-adult viability	$F_{1,13}=5.9*$	$F_{1,13}=0.92$	−	$F_{1,12}=14.52**$	$F_{1,12}=0.71$	−
Female dry weight	$F_{1,13}=3.89†$	$F_{1,13}=29.44***$	−	$F_{1,12}=6.17*$	$F_{1,12}=12.67**$	−

$†P < 0.08$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; all remaining $P > 0.1$