Transgenerational Effects of Parental Larval Diet on Offspring Development Time, Adult Body Size and Pathogen Resistance in *Drosophila melanogaster*

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**Abstract**

Environmental conditions experienced by parents are increasingly recognized to affect offspring performance. We set out to investigate the effect of parental larval diet on offspring development time, adult body size and adult resistance to the bacterium *Serratia marcescens* in *Drosophila melanogaster*. Flies for the parental generation were raised on either poor or standard diet and then mated in the four possible sex-by-parental diet crosses. Females that were raised on poor food produced larger offspring than females that were raised on standard food. Furthermore, male progeny sired by fathers that were raised on poor food were larger than male progeny sired by males raised on standard food. Development times were shortest for offspring whose one parent (mother or the father) was raised on standard and the other parent on poor food and longest for offspring whose parents both were raised on poor food. No evidence for transgenerational effects of parental diet on offspring disease resistance was found. Although paternal effects have been previously demonstrated in *D. melanogaster*, no earlier studies have investigated male-mediated transgenerational effects of diet in this species. The results highlight the importance of not only considering the relative contribution each parental sex has on progeny performance but also the combined effects that the two sexes may have on offspring performance.

**Introduction**

Since phenotypic development is the result of a complex interplay between the genetic architecture of an organism and the environment it experiences during development, a given genotype can give rise to a variety of phenotypes depending on the environmental conditions [1]. In addition to direct environmental effects current and past environmental conditions experienced by other individuals, often the parent(s), may be important in shaping an organism’s phenotype [2]. In fact, it has been suggested that past environmental circumstances may contribute as much as present conditions to variation in current performance [3]. Parental effect is defined as any effect on offspring phenotype that is not determined by the offspring’s DNA but instead is brought about by the genotype or environmental experience of its parents [4,5]. Parents that acquire high condition from a resource-rich environment may benefit by transferring their condition to their offspring, which due to their higher quality will do better under any environmental conditions than offspring of poor-quality parents [2,6]. On the other hand, parents may also respond to environmental cues in ways that enhance offspring performance under particular environmental circumstances. Under this scenario, offspring will do best in an environment similar to that experienced by their parents [2,7].

Variation in parental nutrient provisioning is considered particularly important in shaping offspring phenotype [4]. Whereas diet restriction and mild starvation are often associated with increased longevity and stress tolerance [8–11] poor nutrition during early development is generally associated with negative effects on many adult traits such as body size, survival, secondary sexual trait expression, stress and disease resistance [12–20]. Even if a malnourished individual appears to recover from the nutritional deprivation when food conditions improve, nutritional deficits experienced during key periods of development may have permanent effects on the adult individual and even on its offspring [6,13,21,22]. The complex effect of diet on individual performance is further demonstrated by the growing number of studies showing interaction between parental and offspring nutrition in their effect on offspring performance [6,17,22–26]. Maternal effects are typically considered more important than paternal effects due to the tendency for mothers to invest more resources in production and care of offspring [2,27–30]. The effect of maternal nutrient provisioning on offspring condition and life-history has been documented for a number of species including many insects [2,4,31,32]. Although paternal effects have been reported in species where males contribute to offspring care or provide females with nutrition or other substances that can be transferred to eggs/embryos by the female [4,32–40] paternal effects are often assumed to be mediated solely by the mother when males do not partake in progeny care in the conventional sense [24,40,41]. Recent studies showing transgenerational epigenetic effects have however started to question the relevance of this assumption [42,43].
One such species where males make no obvious material contribution to offspring is Drosophila melanogaster [44]. Even though it is used extensively for studies of nutrition-related life-history trade-offs relatively little is known about cross-generational dietary effects in this species [23]. Whereas maternal dietary effects have been previously described in D. melanogaster [6,23] no data exist for paternal dietary effects. In D. melanogaster, several studies have described paternal effects of temperature and light regimes on a variety of traits including development time and density sensitivity [45], early fecundity [46], cold tolerance [47] and egg size [48]. Furthermore, in a recently published paper by Fréberg et al. [43] substantial variation in egg-to-adult survival owing to paternal effects was uncovered in this species. Transgenerational epigenetic effects were suggested as the most feasible candidate for the observed paternal effects. In mice and in the fly Telostylinus angusticollis dietary effects of both mothers and fathers have been shown to be transmissible to the next generation [24,41]. Because only a few studies have actually tested for environmentally induced paternal effects in species that lack direct paternal investment, the effect of the paternal environment or the potential for joint effects of both parental environments on offspring performance remain poorly understood in such species [24].

In vertebrates offspring can inherit maternal immune function through antibodies [49]. Similar phenomena have recently been observed among invertebrates that rely solely on innate immunity for defense against infection [50–53]. In transgenerational immunity, both the mother and her environment have been shown to influence the phenotype of the offspring. For example, female Daphnia that reproduced under poor nutritional conditions were found to produce offspring that were more resistant to a bacterial pathogen than offspring of mothers that reproduced in a high-food environment [54]. In invertebrates, studies on transgenerational priming have thus far focused mainly on a transfer of antibodies [49]. Similar phenomena have recently been observed among invertebrates that rely solely on innate immunity for defense against infection [50–53]. In transgenerational immunity, both the mother and her environment have been shown to influence the phenotype of the offspring. For example, female Daphnia that reproduced under poor nutritional conditions were found to produce offspring that were more resistant to a bacterial pathogen than offspring of mothers that reproduced in a high-food environment [54]. In invertebrates, studies on transgenerational priming have thus far focused mainly on a transfer of antibodies [49]. Similar phenomena have recently been observed among invertebrates that rely solely on innate immunity for defense against infection [50–53]. In transgenerational immunity, both the mother and her environment have been shown to influence the phenotype of the offspring. For example, female Daphnia that reproduced under poor nutritional conditions were found to produce offspring that were more resistant to a bacterial pathogen than offspring of mothers that reproduced in a high-food environment [54].

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In the present study we aimed to detect any transgenerational effects of paternal early nutrition on offspring development time, adult body size and adult susceptibility to the bacterium Serratia marcescens in Drosophila melanogaster. We only manipulated the parental larval diet, with all adults being placed on standard food on the day they emerged from their puparia. We tested for both maternal and paternal dietary effects as well as for their interaction on offspring raised themselves under standard nutritional conditions. The results of the present study demonstrate the importance of not only considering the relative contribution each parental sex has on progeny performance but also the combined effects that the two sexes may have on offspring performance.

Materials and Methods

Flies and husbandry

Flies (D. melanogaster) used in the experiment were collected from a laboratory base population that had been maintained in the laboratory at room temperature (25 ± 1°C) for approximately four years before the study commenced. Stock larvae are reared on: 10 g agar, 80 g cornmeal, 20 g brewer’s yeast, 1.5 dl syrup, 10 ml nipagin, 1 L water diet (henceforth referred as standard food/standard recipe) and adult flies are fed baker’s yeast. The stock originates from approximately 500 females collected by baits from an apple grove at Lappi in Southern Finland in September 2006. Since their establishment in the laboratory the stock has been expanded and maintained in large glass jars with a standing adult population of several thousand individuals.

Breeding design and development time

For the present study several hundred individuals (400♂, 400♀) were collected as virgin from the stock. At the age of 4–5 days post eclosion the flies were released in a cage and allowed to mate and lay eggs on baker’s yeast supplemented petri dishes for 24 hours. The following day eggs were harvested and transferred either into ‘standard food’ or ‘poor food’ vials at a density of 20 eggs per vial (altogether 50 vials per condition). The ‘standard food’ vials contained 15 ml standard food for the larvae. The ‘poor food’ vials also contained 15 ml standard food but the amount of brewer’s yeast was reduced to 1/3 of the standard amount [19,57–59]. The vials were maintained at 22°C in a 12 L: 12D light regime. As adults emerged (parental generation) they were collected as virgins, housed in same sex groups of 5–8 individuals in vials supplemented with baker’s yeast and at the age of 4–5 days post eclosion crossed in the four possible sex-by-development nutrition combinations:

- males on standard food × females on standard food (S-S, 75 pairs)
- males on poor food × females on poor food (P-P, 74 pairs)
- males on standard food × females on poor food (S-P, 72 pairs)
- males on poor food × females on standard food (P-S, 75 pairs)

The pairs were allowed to interact and lay eggs for 24 hours in 30 ml vials supplemented with baker’s yeast to enhance egg laying (one pair in each vial). The following day eggs were harvested and transferred into ‘standard food’ vials at a density of 20 eggs per vial (20 eggs from each pair in one vial) and placed at 22°C in a 12 L:12D light regime. Development time of the next generation flies was measured as the length of time between ovioposition and adult eclosion. To measure the development time the emerged adults were collected three times a day until eclosion ceased. To ensure virginity, the flies were collected as virgin and housed in same sex groups of 5–8 individuals in vials supplemented with baker’s yeast. Half of the adult flies in each vial were subsequently assigned for the immunity assay; the other half was used as a control (see below). Ice and CO₂ were used in handling the flies.

Pathogen resistance

In the immunity assay survival against a Gram-negative entomopathogenic bacterium Serratia marcescens was measured. The immunity assay was performed on adult flies aged between 5–7 days (post eclosion) and it was carried out in three sets during three successive days (henceforth referred to as experiment day). To measure the strength of immunity towards the bacterium, flies were anesthetized with CO₂, placed on ice, and the thoraces of individual flies pierced with a 0.1 mm pin dipped in a suspension of an overnight culture of the bacteria in liquid broth (OD₆₀₀ = 0.039, LB = 10 g tryptone, 5 g yeast extract and 10 g NaCl, 1 L water). After infection, the flies (females: n_S_S = 271, n_P_P = 218, n_S_P = 238, n_P_S = 219; males: n_S_S = 244, n_P_P = 157, n_S_P = 230, n_P_S = 207) were placed on fresh food and housed in same sex groups of 2–5 individuals at room
temperature (23 ± 1°C). Our previous studies [59] have shown that control flies only pricked with a pin dipped in liquid broth (10 g tryptone, 5 g yeast extract and 10 g NaCl, 1 L water) survive the assay period and hence, in this experiment the control flies (females: n_{S-S} = 278, n_{P-P} = 228, n_{S-P} = 245, n_{P-S} = 231; males: n_{S-S} = 244, n_{P-P} = 164, n_{S-P} = 294, n_{P-S} = 208) were only considered to have survived the treatment. The outline of the bacterial infection follows the assay used by Lazzaro et al. [60,61] and Valtosen et al. [59,62].

**Adult size**

Flies that were used in the immunity assay as control flies as well as those extra individuals that were reserved for the immunity assay but that were not needed in the assay after all were subsequently assigned for the body size assay. Adult body size (thorax length) was measured under a light microscope using an ocular micrometer. Because a large portion of the flies that did not survive the bacterial infection were too fragile to be handled, the infected group of individuals was not measured (neither the ones that survived the infection nor the ones that did not) and hence, we do not have size data for the infected group of flies. However, because the flies were randomly assigned for either the bacterial exposure or the control group (see above) and because of the rather large number of measured flies (altogether 895 females, 794 males) we can be fairly confident that the data gives a realistic picture of the size distribution among the flies in general.

**Statistical methods**

Prior to statistical analysis an average offspring body size and an average offspring development time was calculated for each parental pair (i.e. vial means for males and females) to avoid pseudoreplication. The effect of parental diet on offspring size (vial means) was analyzed using the univariate analysis of variance, with maternal diet, paternal diet and sex as fixed factors. Whereas a statistically significant effect of maternal diet on adult body size was found in both male and female offspring, a statistically significant paternal effect on body size was detected only among male offspring (Table 2). According to the results females were larger than males. Females raised on poor diet produced larger offspring than females that were raised on good diet, paternal diet and sex and all possible two- and three-way interactions terms between these variables were included as covariates in the model. In the model maternal diet, paternal diet and sex were presented as categorical covariates and development time (vial means) as the dependent variable.

**Results**

**Adult size**

Sex and maternal diet had a statistically significant effect on offspring body size. The effect of rearing vial, treated as a random factor, was also significant and the paternal diet×sex interaction was marginally significant (Table 1). To elucidate the meaning of the interaction term, ANOVA was run again, but this time separately for males and females with maternal diet and paternal diet as fixed factors. Whereas a statistically significant effect of maternal diet on adult body size was found in both male and female offspring, a statistically significant paternal effect on body size was detected only among male offspring (Table 2). According to the results females were larger than males. Females raised on poor diet produced larger offspring than females that were raised on good diet, paternal diet and sex and all possible two- and three-way interactions terms between these variables were included as covariates in the model.

**Table 1. Summary of analysis of variance on offspring body size (thorax length) with data pooled over sexes. Significant effects are shown in bold.**

| Source of variation                  | df | Type I SS | MS      | F      | P      |
|-------------------------------------|----|-----------|---------|--------|--------|
| Maternal diet                       | 1  | 0.042     | 0.042   | 10.34  | 0.001  |
| Paternal diet                       | 1  | 0.010     | 0.010   | 2.39   | 0.124  |
| Sex                                 | 1  | 5.564     | 5.564   | 3452.03| <0.001 |
| Maternal diet × Paternal diet        | 1  | 0.007     | 0.007   | 1.83   | 0.178  |
| Maternal diet × Sex                 | 1  | 6×10⁻⁵    | 6×10⁻⁵  | 0.04   | 0.848  |
| Paternal diet × Sex                 | 1  | 0.006     | 0.006   | 3.45   | 0.064  |
| Maternal diet × Paternal diet × Sex  | 1  | 0.001     | 0.001   | 0.74   | 0.389  |
| Vial                               | 252| 1.011     | 0.004   | 2.63   | <0.001 |

*Error term used for the test of significance: SS = 1.007, df = 246.321.
**Error term used for the test of significance: SS = 1.007, df = 246.470.
*Error term used for the test of significance: SS = 0.423, df = 262.208.
**Error term used for the test of significance: SS = 0.423, df = 262.097.
*Error term used for the test of significance: SS = 0.427, df = 264.103.
**Error term used for the test of significance: SS = 0.428, df = 264.718.
*Error term used for the test of significance: SS = 0.337, df = 221.
on standard diet (Figure 1). Accordingly, males raised on poor diet sired larger sons than males that were raised on standard diet (Figure 2).

Development time

Maternal diet, paternal diet, sex and the two-way interaction term paternal diet × maternal diet were included in the model as statistically significant variables predicting development time (Table 3). A significant interaction between the maternal and the paternal diets indicates that a parent’s dietary effect on offspring development time was dependent upon the dietary effect of the other parent. According to the results females developed faster than males. Since the effects of parental diet on offspring development time were independent of sex further analyses (Kaplan-Mayer survival analysis) were conducted on data pooled across sexes. It appears that the progeny of P-P parents had the longest development times, those of S-S intermediate development times and those of S-P and P-S parents had the shortest development times (Figure 3). All comparisons were statistically significant except for that between the progeny of S-P and P-S parents (Table 4).

Pathogen resistance

Disease treatment and the three-way interactions terms maternal diet × paternal diet × treatment and paternal diet × sex × treatment were included as statistically significant variables in the model (Table 5). According to the results survival was worse among the disease treated flies than among the control flies. To better understand the results binary logistic regression analysis was

Table 2. Summary of analysis of variance on offspring body size (thorax length) separately for males and females. Significant effects are shown in bold.

|          | df | Type I SS | MS   | F     | P     |
|----------|----|-----------|------|-------|-------|
| Males    |    |           |      |       |       |
| Maternal diet | 1  | 0.017     | 0.017| 5.59  | 0.019 |
| Paternal diet | 1  | 0.012     | 0.012| 4.13  | 0.043 |
| Maternal diet × Paternal diet | 1  | 0.007     | 0.007| 2.47  | 0.117 |
| Error    | 234| 0.703     | 0.003|       |       |
| Females  |    |           |      |       |       |
| Maternal diet | 1  | 0.015     | 0.015| 5.70  | 0.018 |
| Paternal diet | 1  | 6 × 10⁻⁶ | 6 × 10⁻⁶| 0.02  | 0.879 |
| Maternal diet × Paternal diet | 1  | 0.001     | 0.001| 0.53  | 0.468 |
| Error    | 239| 0.645     | 0.003|       |       |

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Figure 1. Mean body size (thorax length) of female and male offspring. Females raised on a poor diet produced larger offspring than females that were raised on a standard diet.
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Figure 2. Mean body size (thorax length) of female and male offspring. Males raised on a poor diet produced larger sons than males raised on a standard diet. No effect of paternal diet on female body size was detected.
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applied separately for the control and disease treatments. This time, the main effects: parental pair (rearing vial), experiment day, sex, maternal diet and paternal diet, two-way interactions: paternal diet × maternal diet, sex × maternal diet and sex × paternal diet, and a three-way interaction: sex × paternal diet × maternal diet were included as covariates in the model. Among the progeny of S-P and P-S parents. Curves were calculated using the Kaplan-Mayer survival analysis. A significant interaction between the maternal and the paternal diets indicates that a parent’s dietary effect on offspring development time was dependent upon the dietary effect of the other parent. doi:10.1371/journal.pone.0031611.t003

Table 3. Development time was analyzed using Cox regression survival analysis.

|                          | OR  | Wald | df | P    |
|--------------------------|-----|------|----|------|
| Sex                      | 0.511 | 63.629 | 1  | <0.001 |
| Maternal diet            | 1.311 | 10.731 | 1  | 0.001 |
| Paternal diet            | 1.645 | 35.108 | 1  | 0.001 |
| Maternal diet × Paternal diet | 0.078 | 192.603 | 1  | <0.001 |
| Maternal diet × Sex      | 1.078 | 0.208  | 1  | 0.649 |
| Paternal diet × Sex      | 0.867 | 0.737  | 1  | 0.391 |
| Maternal diet × Paternal diet × Sex | 1.744 | 2.818 | 1  | 0.093 |

A significant interaction between the maternal and the paternal diets indicates that a parent’s dietary effect on offspring development time was dependent upon the dietary effect of the other parent.

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Discussion

According to our results offspring whose mothers were raised on poor food as larvae were larger than offspring whose mothers were raised on standard food. Furthermore, male progeny sired by fathers that were raised on poor food were larger than male progeny sired by males raised on standard food. No effect of paternal diet on adult body size of the female offspring was detected. Egg-to-adult development times were shortest for offspring whose one parent was raised on standard and the other parent on poor food (P-S, S-P). Offspring whose parents were raised on standard food (S-S) had intermediate development times. The longest development times were found among offspring whose parents both had experienced poor nutritional conditions as larvae (P-P). No evidence for transgenerational effects of parental nutrition on offspring disease resistance was found.

Transgenerational effects of parental early nutrition

According to life-history theory natural selection could be expected to favor parents that produce fewer but better provisioned offspring in response to cues indicative that offspring will experience nutritional stress [63,64]. In organisms that lack parental care, egg or newborn size can be used as an estimate of parental provisioning [65]. Two studies have previously investigated the effect of maternal diet on offspring performance in D. melanogaster [6,23]. According to Vijendravarma et al. [6] D. melanogaster females raised on poor larval food laid heavier eggs than females raised on standard food which, according to the authors, could indicate enhanced egg provisioning by poorly fed mothers. Moreover, offspring raised on poor food were found to develop faster and be lighter if their mothers also developed on poor food. The extent to which the faster development of offspring of parents raised on poor food was due to the larger egg size rather than maternal effects mediated otherwise was not determined. It was suggested by the authors that although maternal history of poor nutrition may have adverse effects on some traits, adaptive plastic responses on other traits may act to alleviate these negative effects. For some traits the plastic response may even be strong enough for the offspring of the poorly-fed mothers to perform better under poor nutritional conditions than the offspring of well-fed mothers [6]. In contrast to our results, no effect of maternal diet on development time and body size was detected when the offspring were raised on standard food [6]. In a study by Prasad et al. [23] poorly nourished D. melanogaster mothers also showed a tendency of laying heavier eggs than well fed mothers, however, no effect of maternal diet on offspring dry weight at eclosion was observed. Although maternal effects have been previously demonstrated in D. melanogaster [45–48] no earlier studies have investigated male-mediated transgenerational effects of diet in this species. In the fly T. angusticollis (also a species in which there is no evidence of paternal provisioning) variation in the larval diet quality has however been shown to be transmitted across generations through maternal and paternal effects [24]. In this species both mothers and fathers were found to transfer their condition to their offspring, but with effects on different offspring traits [24].

Azevedo et al. [66] studied the effects of egg size on offspring fitness components in D. melanogaster and found that although egg size had a positive effect on hatching weight and development time it had no consistent effects on adult weight. In the light of the above mentioned studies in D. melanogaster it appears unlikely that the observed larger adult size of offspring born to mothers raised on poor larval food would be the result of an enhanced egg provisioning by poorly fed mothers. Moreover, since in insects,
Hence, by directly affecting one of the two traits, development reflects a trade-off with the slower development of these offspring larger size of offspring whose parents were raised on poor food developing to adulthood fast [65,67–69], it is possible that the evolution of larval growth rate and adult body is shaped by the history theory [63,64]. On the other hand, since it is believed that this species have reported (see above) [6,23]. Moreover, it would enhance egg provisioning by parents raised on standard food. offspring born to parents raised on standard food would indicate shorter development [66], the observed faster development of offspring whose parents were raised on poor food provisioning by parents raised on standard food. This would, however, be in contrast to what previous studies in including D. melanogaster, larger egg size is typically associated with shorter development [66], the observed faster development of offspring born to parents raised on standard food would indicate enhanced egg provisioning by parents raised on standard food. This would, however, be in contrast to what previous studies in this species have reported (see above) [6,23]. Moreover, it would not be consistent with the adaptive response predicted by life-history theory [63,64]. On the other hand, since it is believed that the evolution of larval growth rate and adult body is shaped by the tradeoff between the fitness benefits of being large versus those of developing to adulthood fast [65,67–69], it is possible that the larger size of offspring whose parents were raised on poor food reflects a trade-off with the slower development of these offspring (i.e. due to the slow development of offspring of P-P parents). Hence, by directly affecting one of the two traits, development time or adult size, parental nutrition could have caused indirect changes in the other trait.

Our results are similar to those reported by Vijendravarma et al. [6] in that parental dietary effects would seem to involve both adaptive as well as maladaptive effects on offspring performance. According to the results of our study dietary effects of both mothers and fathers can however be transmitted to the next generation and, such effects can be found when the offspring are raised on standard food. The results of the present study could suggest that under appropriate nutritional conditions an individual’s life-history strategy may, at least to some extent, be determined by the nutritional history of its parents. Consequently, when raised under standard nutritional conditions offspring whose parents were raised on standard food would develop faster but be smaller as adults than offspring whose parents were raised on poor food; offspring whose parents have a history of malnourishment would have the opposite strategy. Which of the two life-history

### Table 4. Kaplan-Meier survival analysis was used for the comparisons of development times.

| Parents | \( \chi^2 \) | df | \( P \) |
|---------|-------------|----|-------|
| S-S vs. P-S | 31.732 | 1 | <0.001 |
| S-S vs. P-P | 48.764 | 1 | <0.001 |
| S-S vs. P-P | 47.839 | 1 | <0.001 |
| P-S vs. S-P | 1.446 | 1 | 0.229 |
| P-S vs. S-P | 160.340 | 1 | <0.001 |
| S-P vs. P-P | 191.372 | 1 | <0.001 |

A reduced probability value of \( P = 0.05/6 = 0.008 \) was used to control for multiple comparisons. All comparisons were statistically significant except for that between the progeny of S-P and P-S parents. Log Rank (Mantel-Cox) statistics are reported. doi:10.1371/journal.pone.0031611.t004

### Table 5. Binary logistic regression analysis was used to identify factors associated with pathogen resistance.

| OR | \( \text{Wald} \) | df | \( P \) |
|----|--------------|----|-------|
| Vial | 283.372 | 249 | 0.066 |
| Maternal diet | \( 8 \times 10^{-9} \) | \( 3 \times 10^{-8} \) | 1 | 0.999 |
| Paternal diet | 0.225 | \( 1 \times 10^{-8} \) | 1 | 1.000 |
| Sex | 1.029 | 0.032 | 1 | 0.858 |
| Experiment day | 0.844 | 2 | 0.656 |
| Treatment | 0.050 | 224.070 | 1 | <0.001 |
| Maternal diet x Treatment | 0.801 | 0.310 | 1 | 0.578 |
| Paternal diet x Treatment | 0.750 | 0.515 | 1 | 0.473 |
| Sex x Treatment | 0.892 | 0.128 | 1 | 0.721 |
| Maternal diet x Paternal diet x Treatment | 0.037 | 17.115 | 1 | <0.001 |
| Maternal diet x Sex x Treatment | 0.829 | 0.158 | 1 | 0.691 |
| Paternal diet x Sex x Treatment | 3.054 | 5.606 | 1 | 0.018 |
| Maternal diet x Paternal diet x Sex x Treatment | 1.722 | 0.315 | 1 | 0.575 |

Survival among the disease treated flies was worse than among the control flies. Overall percentage of cases correctly classified by the model: 86.1%. Omnibus Tests of Model coefficients: \( P < 0.001 \). Hosmer-Lemeshow Goodness of Fit Test: \( P = 0.039 \). Nagelkerke R Square: 0.418. doi:10.1371/journal.pone.0031611.t005

### Table 6. Binary logistic regression analysis was used to identify factors associated with pathogen resistance (disease treatment).

| Disease-treatment | OR | \( \text{Wald} \) | df | \( P \) |
|-------------------|----|--------------|----|-------|
| Maternal diet | \( 2 \times 10^{-9} \) | \( 4 \times 10^{-9} \) | 1 | 0.998 |
| Paternal diet | 0.349 | \( 1 \times 10^{-9} \) | 1 | 1.000 |
| Sex | 0.974 | 0.041 | 1 | 0.839 |
| Experiment day | 0.873 | 2 | 0.646 |
| Vial | 192.713 | 248 | 0.996 |
| Maternal diet x Paternal diet | 0.958 | \( 4 \times 10^{-12} \) | 1 | 1.000 |
| Maternal diet x Sex | 0.987 | 0.002 | 1 | 0.961 |
| Paternal diet x Sex | 1.567 | 3.022 | 1 | 0.082 |
| Maternal diet x Paternal diet x Sex | 0.889 | 0.052 | 1 | 0.819 |

Overall percentage of cases correctly classified by the model: 77.5%. Omnibus Tests of Model coefficients: \( P < 0.001 \). Hosmer-Lemeshow Goodness of Fit Test: \( P = 0.675 \). Nagelkerke R Square: 0.322. doi:10.1371/journal.pone.0031611.t006

### Table 7. Binary logistic regression analysis was used to identify factors associated with pathogen resistance (control-treatment).

| Control-treatment | OR | \( \text{Wald} \) | df | \( P \) |
|-------------------|----|--------------|----|-------|
| Maternal diet | 1.466 | \( 6 \times 10^{-10} \) | 1 | 1.000 |
| Paternal diet | 0.928 | \( 1 \times 10^{-11} \) | 1 | 1.000 |
| Sex | 0.995 | \( 1 \times 10^{-4} \) | 1 | 0.992 |
| Experiment day | \( 6 \times 10^{-12} \) | 2 | 1.000 |
| Vial | 28.120 | 248 | 1.000 |
| Maternal diet x Paternal diet | 1.351 | \( 9 \times 10^{-11} \) | 1 | 1.000 |
| Maternal diet x Sex | 1.309 | 0.066 | 1 | 0.797 |
| Paternal diet x Sex | 0.055 | 7.724 | 1 | 0.005 |
| Maternal diet x Paternal diet x Sex | 0.084 | 1.407 | 1 | 0.236 |

Overall percentage of cases correctly classified by the model: 97.4%. Omnibus Tests of Model coefficients: \( P < 0.001 \). Hosmer-Lemeshow Goodness of Fit Test: \( P = 0.781 \). Nagelkerke R Square: 0.686. doi:10.1371/journal.pone.0031611.t007
strategies is most beneficial under the given circumstances cannot be identified by our experimental setup.

**Possible fitness consequences of parental effects**

By comparing development times of offspring of P-P parents with those of S-S parents it would appear that parents transferred their condition to their offspring. However, because the shortest development times were found among offspring whose one parent was raised on standard and the other parent on poor food (P-S, S-P) the mechanistic basis appear more complicated than that. Although the fitness benefits of developing to adulthood fast may be more apparent in the wild where the larval food sources of *D. melanogaster* (decaying fruit) are likely to become unsuitable over time, the larval nutritional environment in the laboratory is also likely to deteriorate with time as the resources are used up by competing larvae and due to the accumulation of waste products. Parental effects on offspring performance have been suggested to be most important when poor environmental conditions are encountered by juveniles [6,24,70]. Being able to develop fast could indeed be particularly advantageous when larvae are developing under adverse nutritional conditions, where development is generally slow [6].

The fitness benefits of developing to adulthood fast and those of being large often trade off with each other [63,67–69]. Whether the observed parental effects on offspring size are sufficient to affect offspring fitness was not determined by us. Because in invertebrates, including *D. melanogaster*, body size is often positively correlated with female fecundity and male mating success [67–69], there are strong grounds for suspecting that regardless of their slower development offspring of parents raised on poor diet would have some fitness advantages due to their larger size. According to Monaghan [71] phenotypic changes that take place during development in response to environmental cues but where the advantage of the induced phenotype is not apparent until later in life should not however be costly in the juvenile stages, otherwise they would be selected against because the forces of selection are likely to be stronger in the younger stages. If the life-history strategies determined by parental effects are fixed, the advantages of adopting a particular life-history strategy will most probably depend on the prevailing environmental conditions. Further studies investigating parental effects under a full set of environmental crossovers between parental and offspring environments are needed to reveal whether parental nutrition really sets patterns of resource allocation in the offspring and whether such effects are sufficient to limit the offspring’s ability to respond to new conditions.

**Paternal effects and the evolution of female mate choice**

In the present study both maternal and paternal dietary effects on offspring size were detected. Whereas the effect of maternal nutrition on offspring size was independent of sex, paternal diet only affected the size of the male offspring. In *D. melanogaster* the advantage of larger males in competition for mates is rather well documented [72–74]. In species lacking conventional forms of paternal provisioning sire effects have been implicated an interesting role in the evolution of female mating preferences [24]. When females prefer to mate with ‘attractive’ males (often those with elaborate secondary-sexual characteristics) but do not receive direct benefits from their mate-choice behavior, it is surmised that females gain indirect genetic benefits from their choice [75]. Over time, persistent female preference for attractive males should however erode genetic variance in the characteristics that the female preference is based upon and eventually, the benefits associated with the preferences would be lost. Nonetheless, female preferences for these traits seem to persist in many taxa [75]. Since purely environmental variation will continue to affect phenotypically plastic traits regardless of genetic variance, it has been suggested that if environmental variation in paternal condition could be transmitted to offspring through paternal effects it could contribute to indirect selection on female preferences [4].

In the fly *T. angusticollis*, in which a paternal diet effect on offspring body size was observed (see above), large, high condition fathers were found to produce larger offspring and it was shown that this paternal effect was sufficient to increase mating success of male offspring and fecundity of female offspring [24]. Although in the present study maternal effects were somewhat more important than paternal effects in explaining variation in male body size (Table 2), males raised under poor nutritional conditions were found to sire larger sons than males raised on standard food. Whether these effects are sufficient to affect male mating success was not determined. The role of paternal effects, if any, in the evolution of female mating preferences and determining male mating success in *D. melanogaster* needs further investigation.

**Possible mechanisms for the transfer of paternal effects in *D. melanogaster***

While our study demonstrates the importance of not only considering the relative contributions each parental sex has on progeny performance but also the potential interactions that may exist among the sexes it does not address the underlying modes of action. In general, whereas maternal effects comprise a number of phenomena [2,76] the possible factors contributing to paternal effects are less clear. In a study by Giesel et al. [45] the effects of maternal and paternal photoperiod on progeny development time were found to be roughly equal in *D. melanogaster*. According to the authors the effect of paternal photoperiod could only be due to alterations in the character of nuclear genomic information since passage of cytoplasmic elements to progeny via sperm is not known to occur in this species.

*D. melanogaster* has a promiscuous mating system and no parental care. In this species males and females only interact during courtship and copulation. With notable exceptions [48,77], egg volume and size are considered to be determined solely by the maternal genotype in *D. melanogaster* [66]. A male mediated effect of temperature on egg size has however been demonstrated in this species [48, but see 43]. In addition, a recently published paper by Pischelda et al. [77] demonstrates that male *D. melanogaster* vary genetically in their influence on egg size. Although these studies did not identify the underlying mechanistic bases for the observed paternal effects, it was suggested by Pischelda et al. [77] that differential female investment in reproduction based on the perceived quality of the mate or alternatively, variation in the ability of males to manipulate female reproductive investment could explain the results. If variation is directly caused by males, it could, according to Pischelda et al. [77], occur via variation in male seminal proteins [77–79]. In *D. melanogaster* the entire sperm is incorporated into the egg during fertilization and may have functional relevance in the early development [80]. Moreover, genes carried by the sperm, the so called paternal effect genes, work during fertilization and are essential for zygote formation and viability [81]. At mating *D. melanogaster* males transfer both sperm and a cocktail of seminal fluid proteins (Sfps) to their mates [78,79]. According to Markow and Ankey [44] incorporation of nutrients from the male ejaculate does not occur in this species. Although Sfps are rather well characterized in *D. melanogaster* the full set of proteins transferred to females, let alone their functions, have not been defined [79]. In some invertebrate species the

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**Parental Nutrition and Offspring Performance**

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accessory gland proteins, the major components of *D. melanogaster* Sfps, have been suggested to play a role in mediating transgenerational parental effects [30–40]. According to a study by Frick et al. [82] the magnitude of female *D. melanogaster* response to a specific ejaculate component, the sex peptide, is significantly affected by the adaptive environment (variation in the amount of yeast provided) in which adult females are maintained. Hence, paternal effects if they are proven to occur via seminal proteins in *D. melanogaster* could, at least in theory, also be affected by nutritional conditions experienced by the parents. Moreover, epigenetic modifications of sperm DNA could play a role in paternal transmission of dietary effects [41,42]. In a recently published paper by Friberg et al. [43] transgenerational epigenetic effects were indeed considered the most feasible candidate for the paternal effects on egg-to-adult survival found in *D. melanogaster*. The occurrence of paternal effects in species where there is no paternal care suggests that the fertilizing sperm has more function than hitherto thought [38–41,45–48].

**Conclusions**

Past environmental conditions, especially those experienced by the mother, are considered important in shaping offspring phenotype, moreover, they have been shown to play an important role in determining the way offspring respond to current environmental conditions [6,17,22–26]. The extent to which maternal environment influences offspring phenotype and fitness is considered to determine whether such effects themselves will be indicated on by natural selection [2]. The existence of paternal effects indicates that paternal experience may also be translated into variation in offspring fitness. In addition to their practical significance such effects would have important theoretical implications in the field of quantitative genetics for their potential to inflate estimates of additive genetic variance [43]. The emerging evidence supporting the occurrence of paternal effects in species with no paternal care suggests that such effects are far more common than hitherto appreciated. Whether parental effects are independent of the mate, or whether parental effects generally change depending on the combination of the parental phenotypes need further investigation. In order to be able to generalize, this work must include species from multiple taxa.

In conclusion, this work highlights the importance of not only considering the relative contribution each parental sex has on progeny performance but also the possibility of their joint effects. Furthermore, the results of the present study suggest that under appropriate nutritional conditions an individual's life-history strategy may be set by the nutritional history of its parents. Further work is required to investigate whether such life-history strategies are fixed or can be overcome with a change in diet. In the present study, we studied the relationship between parental early nutrition and one component of invertebrate immunity and observed no effect. Since different components of the immune system do not necessarily show correlated responses [83], it would be of interest to investigate the effect of parental early nutrition on other aspects of offspring immunity.

**Author Contributions**

Conceived and designed the experiments: MJR TMV. Performed the experiments: TMV KK MP. Analyzed the data: TMV. Contributed reagents/materials/analysis tools: MJR. Wrote the paper: TMV. Supervision of the study: MJR.

**References**

1. West-Eberhard MJ (2005) Developmental plasticity and evolution. Oxford, U.K.: Oxford University Press, 794 p.
2. Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. Trends Ecol Evol 13: 403–407.
3. Deveyve G, Baze P, Fournier S, Person E, Christe P (2010) Testing the predictive adaptive response in a host-parasite system. Funct Ecol 24: 178–185.
4. Bonduriansky R, Day T (2009) Nongenetic inheritance and its evolutionary implications. Annu Rev Ecol Evol Syst 40: 103–125.
5. Youngson NA, Whelan E (2008) Transgenerational epigenetic effects. Annu Rev Genet 9: 233–257.
6. Vijendravarma RK, Narasimha S, Kasvecki TJ (2010) Effects of paternal diet on egg size and offspring traits in *Drosophila*. Biol Lett 6: 238–241.
7. Badyaev AV, Uller T (2009) Parental effects in ecology and evolution: mechanisms, processes and implications. Phil Trans R Soc B 364: 1169–1177.
8. Bahl OA, Ismailova AG, Leerschke V (1990) Selection for knockdown resistance to heat in *Drosophila melanogaster* at high and low larval diet. Evolution 52: 619–625.
9. Wenzel U (2006) Nutrition, surivivum and aging. Genes Nutr 1: 65–93.
10. Burger JMS, Hwangbo DS, Corby-Harris V, Promislow DEL (2007) The functional costs and benefits of dietary restriction in *Drosophila*. Aging Cell 6: 63–71.
11. Smith EM, Hoi JT, Eisenberg JC, Shoemaker JD, Neckameyer WS, et al. (2007) Feeding *Drosophila* a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns. J Nutr 137: 2006–2012.
12. Lindström J (1999) Early development and fitness in birds and mammals. Trends Ecol Evol 14: 345–348.
13. Metcalfe NR, Monaghan P (2003) Compensation for a bad start: growth now, pay later? Trends Ecol Evol 16: 254–260.
14. Lummaa V, Clutton-Brock T (2002) Early development, survival and reproduction in humans. Trends Ecol Evol 17: 141–147.
15. Lakhkanid FD, Hanson MA (2004) Living with the past: evolution, development, and patterns of disease. Science 305: 1733–1736.
16. Waterland RA, Jirtle RL (2004) Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 20: 63–68.
17. Mitchell SE, Read AF (2005) Poor maternal environment enhances offspring disease resistance in an invertebrate. Proc R Soc Lond B 272: 2601–2607.
18. Taborsky B (2006) The influence of juvenile and adult environments on life-history trajectories. Proc R Soc Lond B 273: 741–750.
37. Gillott C (2003) Male accessory gland secretion: modulators of female reproductive physiology and behavior. Annu Rev Entomol 48: 163–184.
38. Garcia-Gonzalez F, Simmons LW (2003) The evolution of polyandry: intrinsic size effects contribute to embryo viability. J Evol Biol 16: 1097–1103.
39. Garcia-Gonzalez F, Simmons LW (2007) Paternal indirect genetic effects on offspring viability and the benefits of polyandry. Curr Biol 17: 32–36.
40. Ivy TM (2007) Good genes, genetic compatibility and the evolution of polyandry: use of the dillil construction to address competing hypotheses. J Evol Biol 20: 479–487.
41. Curley JP, Mashoodh R, Champagne FA (2011) Epigenetics and the origins of parental effects. Horm Behav 59: 306–314.
42. Jablonka E, Raz G (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84: 131–176.
43. Friberg U, Stewart AD, Rice WR (2011) X- and Y-chromosome linked paternal effects on a life-history trait. Biol Lett 7: 10.1098/rsbl.2011.0690.
44. Markow TA, Aukeny PJ (1984) Drosophila males contribute to oogenesis in a multible mating species. Science 224: 302–303.
45. Giesel JT (1988) Effects of parental photoperiod on development time and density sensitivity of progeny of Drosophila melanogaster. Evolution 42: 1748–1750.
46. Huey RB, Wakefield T, Grill WD, Gilchrist GW (1995) Within- and between-generation effects of temperature on early fecundity of Drosophila melanogaster. Heredity 74: 216–223.
47. Watson MJ, Hoffmann AA (1995). Cross-generation effects for cold resistance in tropical populations of Drosophila melanogaster and D. simulans. Aust J Zool 43: 51–58.
48. Crill WD, Huey RB, Gilchrist GW (1996) Within- and between-generation effects of temperature on the morphology and physiology of Drosophila melanogaster. Evolution 50: 1205–1218.
49. Grindstaff JL, Brodie EDI, Jetteson AD (2003) Immune function across generations: integrating mechanisms and evolutionary process in maternal antibody transmission. Proc R Soc Lond B 270: 2309–2311.
50. Little TJ, O'Connor B, Colegrave N, Watt K, Read AF (2003) Maternal transfer of strain-specific immune in an invertebrate. Curr Biol 13: 489–492.
51. Sadik BM, Kleinigob Y, Schmid-Hempel R, Schmid-Hempel P (2005) Trans-generational immune priming of a social insect. Biol Lett 1: 386–388.
52. Moret Y (2006) 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor. Proc R Soc B 273: 1399–1405.
53. Sadik BM, Schmid-Hempel P (2007) Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. Curr Biol 17: R1046–R1047.
54. Mitchell SE, Read AF (2005) Poor maternal environment enhances offspring disease resistance in an invertebrate. Proc R Soc Lond B 272: 2601–2607.
55. Roth O, Joop G, Eggert H, Hilbert J, Daniel J, et al. (2010) Paternally derived immune priming for offspring in the red flour beetle, Tribolium castaneum. J Anim Ecol 79: 403–413.
56. Miller GA, Pell JK, Simpson SJ (2009) Crowded locusts produce hatchlings vulnerable to fungal attack. Biol Lett 5: 845–848.
57. Krebs RA, Loeschcke V (1994) Costs and benefits of activation of the heat-shock response in Drosophila melanogaster. Funct Ecol 8: 730–737.
58. Babity OA, Imashiva AG, Loeschcke V (2000) Half-sib analysis of three Morphological traits in Drosophila melanogaster under poor nutrition. Hereditas 133: 59–63.
59. Valtonen TM, Roff DA, Rantala MJ (2011) Analysis of the effects of early nutritional environment on inbreeding depression in Drosophila melanogaster. J Evol Biol 24: 196–205.
60. Lazzaro BP, Seurman BR, Clark AG (2004) Genetic basis of natural variation in D. melanogaster antibacterial immunity. Science 303: 1873–1876.
61. Lazzaro BP, Sackton TB, Clark AG (2006) Genetic variation in Drosophila melanogaster resistance to infection: a comparison across bacteria. Genetics 174: 1539–1554.
62. Valtonen TM, Kleinso A, Ramet M, Rantala MJ (2010) Starvation reveals maintenance cost of humoral immunity. Evol Biol 37: 49–57.
63. Smith CC, Fretwell SD (1974) Optimal balance between size and number of offspring. Am Nat 108: 499–506.
64. Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol 45: 341–369.
65. Roff DA (2002) Life history evolution. SunderlandMassachusetts: Sinauer Associates. pp 527.
66. Aviseved RBR, French V, Partridge L (1997) Life-history consequences of egg size in Drosophila melanogaster. Am Nat 150: 250–262.
67. Santos M, Ruiz A, Barbashilla A, Quezada-Diaz JE, Hasson E, et al. (1980) The evolutionary history of Drosophila buzzatii. XIV. Larger flies mate more often in nature. Heredity 61: 253–262.
68. Partridge L, Fowler K (1993) Responses and correlated responses to artificial selection on thorax length in Drosophila melanogaster. Evolution 47: 213–226.
69. Joshi A (2004) Are bigger flies always better: the role of genes and environment. J Genet 83: 13–15.
70. Donelson JM, Munday PL, McCormick MJ (2009) Parental effects on offspring life histories when are they important? Biol Lett 5: 262–263.
71. Monaghan P (2006) Early growth conditions, phenotypic development and environmental change. Phil Trans R Soc B 363: 1635–1645.
72. Partridge L, Farquhar M (1983) Lifetime mating success of male fruitflies (Drosophila melanogaster) is related to their size. Anim Behav 31: 871–877.
73. Partridge L, Ewings A, Chandler A (1987) Male size and mating success in Drosophila melanogaster: the roles of male and female behaviour. Anim Behav 35: 552–562.
74. Partridge L, Hoffmann A, Jones JS (1987) Male size and mating success in Drosophila melanogaster and D. pseudoobscura under field conditions. Anim Behav 35: 466–476.
75. Miller CW, Moore AJ (2007) A potential resolution to the lek paradox through indirect genetic effects. Proc R Soc Lond B 274: 1279–1286.
76. Wolf JB, Wade MJ (2009) What are maternal effects (and what are they not)? Phil Trans R Soc B 364: 1107–1115.
77. Pachurda A, Stewart AD, Little MK, Rice WR (2011) Male genotype influences female reproductive investment in Drosophila melanogaster. Proc R Soc B 278: 2165–2172.
78. Chapman T (2001) Seminal fluid-mediated fitness traits in Drosophila. Heredity 87: 511–521.
79. Findlay GD, Yi X, MacCoss MJ, Swanson WJ (2008) Protaxonics reveals novel Drosophila seminal fluid proteins transferred at mating. PLoS Biol 6: e178.
80. Karr TL (1991) Intracellular sperm/egg interactions in Drosophila: a three-dimensional structural analysis of a paternal product in the developing egg. Mech Dev 34: 101–112.
81. Loppin B, Lepeit D, Dorus S, Couble P, Karr TL (2005) Origin and neofunctionalization of a Drosophila paternal effect gene essential for zygote viability. Curr Biol 15: 87–93.
82. Fricke C, Breman A, Chapman T (2010) Female nutritional status determines the magnitude and sign of responses to a male ejaculate in Drosophila melanogaster. J Evol Biol 23: 157–165.
83. Adamo SA (2004) How should behavioural ecologists interpret measurements of immunity? Anni Behav 68: 1443–1449.