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# Idiosyncratic evolution of maternal effects in response to juvenile malnutrition in *Drosophila*

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## Abstract

Maternal effects often affect fitness traits, but there is little experimental evidence pertaining to their contribution to response to selection imposed by novel environments. We studied the evolution of maternal effects in *Drosophila* populations selected for tolerance to chronic larval malnutrition. To this end, we performed pairwise reciprocal $F_1$ crosses between six selected (malnutrition tolerant) populations and six unselected control populations and assessed the effect of cross direction on larval growth and developmental rate, adult weight and egg-to-adult viability expressed under the malnutrition regime. Each pair of reciprocal crosses revealed large maternal effects (possibly including cytoplasmic genetic effects) on at least one trait, but the magnitude, sign and which traits were affected varied among populations. Thus, maternal effects contributed significantly to the response to selection imposed by the malnutrition regime, but these changes were idiosyncratic, suggesting a rugged adaptive landscape. Furthermore, although the selected populations evolved both faster growth and higher viability, the maternal effects on growth rate and viability were negatively correlated across populations. Thus, genes mediating maternal effects can evolve to partially counteract the response to selection mediated by the effects of alleles on their own carriers' phenotype, and maternal effects may contribute to evolutionary trade-offs between components of offspring fitness.

## Introduction

The ability of individuals to cope with environmental stress, in particular at an early age, is often subject to parental effects, defined as the causal influence of parental (in particular maternal) environment or genotype on offspring phenotype. In the absence of parental care, such effects can be mediated by egg provisioning with nutrients as well as by transcripts, hormones and other signalling molecules deposited in the egg by the mother (Mousseau & Fox, 1998; Wolf & Wade, 2009). The effects of parental environment on stress tolerance are well studied. While in many cases parents exposed to stress produce inferior offspring, there is also evidence for adaptive effects where mothers exposed to a particular stress 'prime' an adaptive plastic response in the offspring that helps them cope with that particular stress (Mousseau & Fox, 1998; Agrawal *et al.*, 1999). Although there is ample evidence for genetic parental effects (i.e. genetic variation expressed in parents that affects offspring performance, reviewed by Räsänen & Kruuk, 2007), we know still relatively little about the contribution of genetic parental effects to evolutionary adaptation to stress and other environmental challenges (Badyaev & Uller, 2009). One might expect that alleles that are expressed in the parents and improve the offspring ability to cope with stress should be favoured by natural selection, in which case the parental effects would evolve synergistically with the response based on allelic variation expressed in the offspring (Hoyle & Ezard, 2012). Several examples of such synergistically evolving maternal effects have been inferred from natural populations adapting to novel environments (Badyaev, 2005; Duckworth, 2009; Hangartner *et al.*, 2012) and observed to contribute to responses to artificial selection (e.g. Goodwill, 1975; Park *et al.*, 2006). However, such alleles might have negative effects on the

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parent's own stress tolerance and thus residual reproductive value, resulting in a trade-off between parent and offspring fitness (Räsänen & Kruuk, 2007; Duckworth, 2009). In such a case, parental effects may evolve in a direction that is antagonistic to the response mediated by genetic variation expressed in the offspring resulting in a parent–offspring conflict (e.g. Teotonio *et al.*, 2004; McGlothlin & Galloway, 2014). Finally, several manipulative experiments show that maternal effects can mediate physiological trade-offs between offspring immunity and growth or fecundity (e.g. Groothuis *et al.*, 2005; Schlotz *et al.*, 2013).

Theory predicts that, in addition to the phenotypic effect on offspring, maternal effects may also facilitate or hinder adaptive evolution based on alleles expressed in the offspring (Kirkpatrick & Lande, 1989; McGlothlin & Galloway, 2014; Prizak *et al.*, 2014). Although this does not require that the traits mediating the maternal effects themselves evolve, the prediction can be considerably affected if these maternal effect traits do evolve (McGlothlin & Galloway, 2014). Therefore, understanding how frequently and in what way genetic variation in maternal effects responds to selection is important for understanding the dynamics of adaptive evolution.

This study focuses on the contribution of maternal effects to adaptation to chronic juvenile malnutrition as an example of an ecologically relevant stress factor. We have previously shown that even though *Drosophila melanogaster* females raised on a low-quality larval food are 30% smaller than females raised on a standard food, they lay 3–6% larger eggs (Vijendravarma *et al.*, 2010). Furthermore, offspring raised on the poor food pupate 4% earlier and emerge 3% smaller if their parents have also been raised on the poor food, compared with offspring of parents raised on the standard food (Vijendravarma *et al.*, 2010; see also Valtonen *et al.*, 2012). These plastic maternal effects are arguably adaptive – they parallel evolutionary changes occurring in populations maintained on the poor food (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2012). Maternal genotype also affects performance of *Drosophila* larvae under malnutrition – a quantitative genetic design revealed that the contribution of genetic maternal effects to variance in larval viability and developmental time on poor food was similar in magnitude to the additive genetic variance component (Nepoux *et al.*, 2015; variance in body weight was not assessed in that study). This implies that maternal effects have the potential to evolve in response to selection imposed by repeated exposure to larval malnutrition. This was confirmed in line-cross analysis between a selected population which, in the course of 84 generations of experimental evolution, adapted to the poor larval food and a control population originally derived from the same base population but maintained on standard food (Vijendravarma & Kawecki, 2013). In particular, roughly half of the substantial increase in larval survival on the poor food shown by the selected population could be attributed to effects of genes expressed in the mother, rather than to effects of genes expressed in the larvae themselves (Vijendravarma & Kawecki, 2013). Evolutionary change in maternal effects also contributed to a reduction in adult body size without reducing developmental time, suggesting that maternal effects evolved in the possibly maladaptive direction of reducing offspring growth rate (Vijendravarma & Kawecki, 2013).

The complex design of Vijendravarma & Kawecki's (2013) study, which involved 14 different first- and second-generation crosses, offered other insights into the genetic architecture of malnutrition tolerance in the selected population, for example finding strong antagonistic epistasis between the favoured alleles for all traits. One of its limitations was its unreplicated nature – because of the size of the crossing design, only the architecture of the difference between one selected (malnutrition-adapted) and one control population was investigated. The two populations crossed in that study were randomly chosen from six replicate selected and six replicate control populations (Kolss *et al.*, 2009). The replicated populations show largely parallel evolutionary responses of life history phenotypes (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2012). However, phenotypically parallel evolution may still rely on different allele substitutions, reflecting drift pushing populations towards alternative 'adaptive peaks' (Whitlock *et al.*, 1995); such idiosyncratic genetic architectures has been detected in some evolution experiments (e.g. Cohan & Hoffman, 1989; Kawecki & Mery, 2006). Therefore, the question remains whether the large contribution of maternal effect to tolerance to chronic malnutrition detected by Vijendravarma & Kawecki (2013) is a regular feature of adaptation to larval malnutrition in *Drosophila* vs. being specific to the evolutionary trajectory of the one evolved population analysed in that paper. We address this question in the present paper by assessing the effect of $F_1$ cross direction on offspring life history traits across the six selected populations adapted to larval malnutrition, crossing each of them to a different control population. Although this simple cross design does not separate maternal effects from the effects of cytoplasmic (i.e. mitochondrial or endosymbiont) genomes, it does allow the assessment of the degree to which such parent-of-origin effects are parallel across independently evolved populations.

## Materials and methods

### The study populations

The history of the study populations and their evolutionary regimes is described in detail elsewhere (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2012). Twelve populations were derived from the same laboratory-adapted outbred base population. Six control populations were

maintained on standard larval food (50 g cornmeal, 30 g sucrose, 60 g glucose, 12.5 g dry yeast, 15 g agar, 0.5 g $MgSO_4$, 0.5 g $CaCl_2$, 30 mL ethanol, 6 mL propionic acid and 1 g nipagin per litre of water); six selected populations were maintained on poor food containing 1/4 of the concentrations of nutrients (i.e. cornmeal, glucose, sucrose and yeast) relative to the standard food. Eclosed adults were collected on day 14 from egg laying, effectively imposing an upper limit on developmental time. In both regimes, adults were fed standard food with additional live yeast; thus, only larvae were exposed to nutritional stress in the selected populations. All experiments were carried out at 25 °C and 50–70% relative humidity under controlled larval density of about 200–250 eggs per 30 mL of food. The census size of each population was regulated at 200 breeding adults. The experiment reported here took place after 103 generations of selection. All populations were maintained for two generations on standard food prior to the experiment to eliminate the effects of maternal environment.

### Reciprocal crosses between selected and control populations

Each selected population was crossed with a different control population, creating six pairs of reciprocal crosses (i.e. control population C1× selected population S1, C2× S2, etc). This is effectively an arbitrary pairing because the populations are not paired in any way by the design of the evolution experiment; C1× S1 is the population pair whose divergence was analysed by Vijendravarma & Kawecki (2013). The procedures followed those used by Vijendravarma & Kawecki (2013). The parental generation was raised under standard conditions in multiple vials per population. Virgin females and males were isolated upon emergence and maintained in single-sex groups on standard food for 4–6 days. Subsequently, we set up 6 replicate mating vials per cross, each with about 25 males and females, on standard food supplemented with live yeast. After 48 h, these mating groups were transferred for 2 h to a fresh oviposition medium to stimulate laying of any previously fertilized and already developing eggs, potentially retained by the females because of declining quality of the medium in the mating vials. The eggs used in the assay were subsequently collected during 4 h. This protocol was used to minimize variation in the timing of fertilization. Two hundred eggs from each mating group were thoroughly washed with water and transferred to a vial containing 30 mL of poor food, resulting in a total of 72 vials (6 pairs of crosses × 2 cross directions × 6 replicate vials). Eclosed adults were counted and sexed every 24 h. Twelve females and twelve males emerged on the day of peak adult emergence (or, if necessary, over two peak days) were collected form each vial, dried at 70 °C over 3 days and weighed as a group to the nearest μg.

### Analysis

Sex-specific egg-to-adult viability was estimated for each vial by dividing the number of surviving individuals of each sex by 100; this assumes a 50 : 50 primary sex ratio. We analysed both untransformed and logit-transformed viability; both analyses yielded very similar results, so we only report the untransformed analysis because its residuals conformed better to normal distribution. An estimate of adult weight of each sex was obtained from the group weight of 12 individuals weighed together (see above). Developmental rate was calculated for each eclosed adult as the inverse of its developmental time and used to calculate the mean developmental rate of males and females for each replicate vial. Differences in body weight are a combined result of difference in growth rate and critical size for metamorphosis (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2012). As an attempt to disentangle these two effects, and following Kolss *et al.* (2009), we derived a rough estimate of growth rate of the larvae, assuming exponential growth. It was calculated as

$$g = ln(w_a/w_e)/T, \quad (1)$$

where $w_a$ is the adult dry weight estimated in the experiment, $w_e$ is egg dry weight estimated at 5 μg (Kolss *et al.*, 2009), and $T$ is the length of larval growth period estimated as egg-to-adult developmental time minus 5 days to account for time needed for egg hatching and metamorphosis. The selected and control larvae do not differ with respect to time spent wandering before pupation (Narasimha *et al.*, 2015) or in the length of the pupal period (R. K. Vijendravarma, unpublished data).

All four traits were analysed with a general linear model, with cross direction, sex and direction × sex as fixed effects, and population pair (i.e., identity of the parental populations) and its interactions with sex as random effects; vial was a random effect nested within cross direction × population pair. (Although a generalized linear model with binomial error distribution would in principle be more appropriate for the viability data, it would not easily accommodate random factors which were important in our design.) Residuals for all traits conformed to a normal distribution (Shapiro–Wilk test, all $P > 0.1$). Because the above analysis detected large interactions involving population pair, we also analysed separately the results for each population pair, with the same fixed factors as above, and with vial nested within cross direction as a random factor. In this case, we used the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995) to control for the six multiple comparisons, assuming false discovery rate of 5%. We followed Scheffe's mixed model for the composition of expected mean squares Snedecor & Cochran, 1967; Ayres & Thomas, 1990.

To interpret the analysis, we note that an effect of cross direction that is similar in both sexes indicates a maternal effect (including an effect of cytoplasmic genomes). Although an interaction between cross direction and sex might result from sex-specific maternal effects, it more parsimonious to attribute them to effects of the X chromosome – X chromosome contains about 20% of *Drosophila* genome and has been found to contribute to divergence between a selected and a control population analysed by Vijendravarma & Kawecki (2013). However, because females receive an X chromosome from each parent, the effect of cross direction on female phenotypes cannot be attributed to the X chromosome and thus implies maternal (and/or cytoplasmic) effect. Therefore, for each population pair, we additionally tested the effect of cross direction on males and females separately. We interpreted a significant main effect of cross direction on a phenotype as evidence for a maternal effect only if there was no cross direction × sex interaction. If such interaction was present, the criterion for detection of a maternal effect was an effect of cross direction on female phenotypes.

## Results

To facilitate verbal description of results, we refer to a positive (negative) effect of cross direction as a shorthand to mean that the cross between a selected mother and control father results in a higher (lower) mean of the focal trait than the cross in the opposite direction. The most striking general result that emerged from the analysis is that the effects of cross direction were ubiquitous but highly idiosyncratic across the pairs of populations (Fig. 1, Table 1). As a consequence, although the main effect of cross direction did not approach significance for any trait, the interaction between cross direction and population pair was highly significant for all traits (Table 1). Below we summarize the patterns for each trait.

Apart from the (expected) effect of sex on developmental rate, adult weight and growth rate, the only

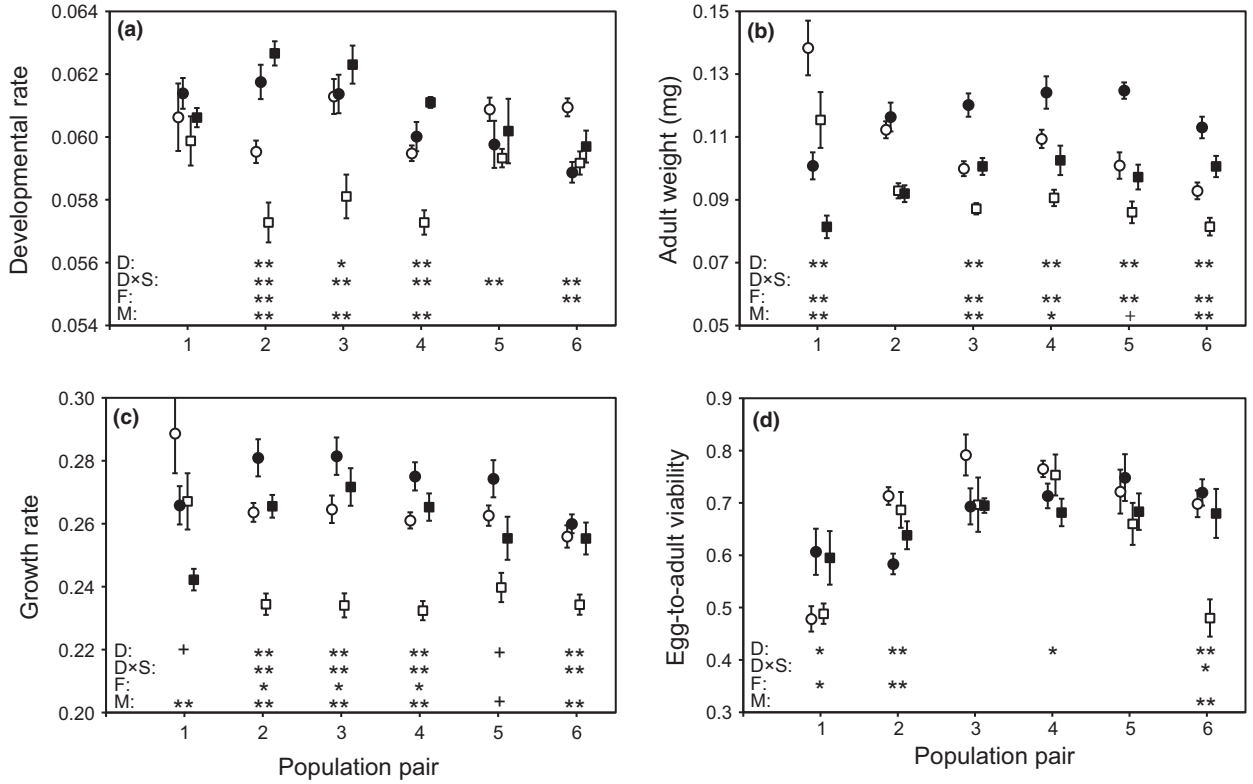


**Fig. 1** The mean phenotypes (± SE) of the reciprocal first-generation crosses between six pairs of Selected and Control populations. (a): developmental rate (inverse of egg-to-adult developmental time in days). (b): Adult dry weight upon eclosion. (c): Average larval growth rate estimated from adult dry weight and developmental time (see Methods). (d): Egg-to-adult viability (proportion of eggs that gave rise to adults, estimated under the assumption of 50 : 50 primary sex ratio). Circles: females; squares: males; open symbols: Control mother × Selected father, closed symbols: Selected mother × Control father. The asterisks at the bottom of each panel indicate the significance of the effects of direction of cross (D), direction × sex interaction (D×S), the contrast between mean phenotypes in females (F) and males (M). ** Benjamini–Hochberg adjusted $P < 0.05$; *nominal $P < 0.05$, adjusted $0.05 < P < 0.1$; $^{+}$nominal and adjusted $0.05 < P < 0.1$.

**Table 1** Summary of analysis of variance (values of *F*-test and significance) for reciprocal first-generation crosses between six pairs of control and selected lines; 'Direction' refers to the direction of the cross (Control × Selected vs. Selected × Control).

| Source | d.f. | Denominator MS | Developmental rate | Weight | Growth rate | Viability |
|---|---|---|---|---|---|---|
| Direction | 1,5 | Direction × pop pair | 3.7 | 0.4 | 2.9 | 0.1 |
| Sex | 1,5 | Sex × pop pair | 52.4*** | 134.9*** | 194.9*** | 3.8 |
| Direction × sex | 1,5 | Dir. × sex × pop. pair | 18.5** | 3.5 | 10.8* | 2.5 |
| Population pair | 5,60 | Vial(dir. × pop. pair) | 2.0† | 2.8* | 2.2† | 17.1*** |
| Direction × pop. pair | 5,60 | Vial(dir. × pop. pair) | 5.3*** | 117.6*** | 7.7*** | 7.2*** |
| Sex × pop. pair | 5,60 | Residual | 0.6 | 2.3† | 1.5 | 2.2† |
| Dir. × sex × pop. pair | 5,60 | Residual | 5.8*** | 1.1 | 2.6* | 1.3 |
| Vial(dir. × pop. pair) | 60,60 | Residual | 6.0*** | 3.7*** | 5.7*** | 0.9 |

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
†$P < 0.1$.

pattern detected across the population pairs was the significant cross direction × sex interaction for developmental rate (Table 1, Fig. 1a). Averaged across the population pairs, males developed about 4.4% faster if their mother was from a selected rather than from a control population, whereas the female developmental rate averaged across population pairs was nearly identical for each cross direction (0.1% difference). This cross direction × sex interaction was detected for five of the six population pairs; it suggests a contribution of genetic changes on the X chromosome to the faster development of larvae from the selected populations. Female developmental rate was affected by cross direction for two population pairs, but in contrasting ways, implying a positive maternal effect in population pair 2 and a negative maternal effect in population pair 6 (Fig. 1a).

For four population pairs (3–6), the offspring of both sexes were heavier if the mother originated from the selected line; for pair 1, the opposite was the case, and for pair 2, the means of reciprocal crosses were nearly identical (Fig. 1b). While idiosyncratic among population pairs, the effects of cross direction were highly consistent between the sexes (no hint of cross direction × sex interaction), implicating maternal rather than X chromosome effects.

In contrast to body weight, growth rate showed a similar pattern of cross direction × sex interaction as developmental rate, implying that the apparent effects of X chromosome on developmental time are mediated through improved growth and not through reduced adult size. Three population pairs (2–4) show some evidence of positive maternal effects on growth rate, with the effect of cross direction on female phenotype being nominally significant (although not significant when adjusted to false discovery rate of 0.05).

For population pair 1, offspring survived better when the mother originated from the selected population (Fig. 1d). Even though this effect would not be significant after correction for multiple comparisons, it provides confirmatory evidence for the additive maternal effect found for this pair of populations by Vijendravarma & Kawecki (2013). Population pair 2 showed the opposite pattern, implying a negative maternal effect on offspring viability; a similar trend was observed for population pair 4. Finally, the results for population pair 6 pointed to a contribution of X chromosome, reflected in a large difference in male viability but nearly identical female viability.

## Discussion

Several main conclusions can be drawn from these results. First, for all population pairs, at least one of the four traits was affected by cross direction in a way implying maternal effects (i.e. parallel effect of cross direction on offspring of both sexes or significant effect on female offspring). These effects were large, often comparable in magnitude to overall evolutionary divergence between the selected and control populations reported before. For example, the 12% difference in female viability between reciprocal crosses in population pair 1 (Fig. 1d) would account for a large portion of the 20% viability difference between these populations reported by Vijendravarma & Kawecki (2013). Similarly, the difference in growth rate between reciprocal crosses in population pairs 2 and 3 (about 0.017) would account for an even larger part of the average difference of about 0.027 in growth rate between the selected and control populations reported by Kolss *et al.* (2009). This implies that experimental evolutionary adaptation to chronic larval malnutrition in all replicate populations was mediated in part by allele substitutions which affected the phenotype via their expression in the mothers of the focal individuals.

Second, while present in all population pairs, the maternal effects were highly idiosyncratic – which traits were affected and in which direction depended on the

specific populations. This suggests that even though the evolutionary changes in response to the malnutrition regime were mostly parallel at the phenotypic level, the underlying genetic and developmental architecture varied among replicate populations. Even if the replicate gene pools are originally sampled from the same base population, the trajectory of adaptive evolution can be affected by drift if the adaptive landscape is 'rugged', involving strong epistatic interactions (Wade & Goodnight, 1998). Furthermore, different populations may evolve the same phenotypic change based on different genetic architectures (Gilchrist *et al.*, 2001). Theoretical arguments and empirical evidence implies that epistatic interactions are particularly ubiquitous for life history traits (Roff & Emerson, 2006), and we found large epistatic effects contributing to divergence between a selected and a control population (Vijendravarma & Kawecki, 2013). Our study thus adds to experimental evidence that even short-term responses to uniform selection from standing genetic variation can follow different trajectories on the genotypic adaptive landscape (Cohan & Hoffman, 1989; Cohan *et al.*, 1989; Kawecki & Mery, 2006; Simoes *et al.*, 2008).

Third, many but not all of these maternal effects can be tentatively interpreted as adaptive under the malnutrition regime under which they evolved. The maternal effect on viability observed in the first population pair (Fig. 1d) directly contributes to offspring fitness. By the same token, the negative effect on viability in population pairs 2 and 4 must reduce offspring fitness (possibly reflecting a trade-off discussed below). In the course of their experimental adaptation to the malnutrition regime, the selected populations also evolved faster development (Kolss *et al.*, 2009); hence, the positive maternal effect on developmental rate in the second population pair (Fig. 1a) is also likely to be adaptive, in particular given that it is mediated by faster growth rate, with no reduction in adult size (Fig. 1b,c). The effects on body weight are less straightforward to interpret. The selected populations evolved smaller adult size, mediated by a smaller critical size at which metamorphosis is initiated (Vijendravarma *et al.*, 2012). However, in *Drosophila* adult fitness components, and in particular female fecundity, are strongly positively correlated with body size (e.g. Robertson, 1957). The smaller adult size in the selected populations is thus probably a price for being able to survive and develop fast under poor and deteriorating food conditions, rather than resulting from direct selection for reduced size (Vijendravarma *et al.*, 2012). Therefore, the positive maternal effect of malnutrition-adapted mothers on offspring weight in population pairs 3–5 is likely adaptive – it is not associated with delayed development, but apparently mediated by faster growth, which presumably reflects a greater efficiency in the use of the scarce nutrients. In contrast, the effect of cross direction on female weight in population pair 6 is associated with slower development, suggesting a maternal effect that is antagonistic to the direction of evolution of these two traits in the selected populations, and thus likely maladaptive. As argued in the Introduction, such maladaptive maternal effects may result from trade-offs with the mother's own viability or fecundity, although we could not address them with this study.

Fourth, our results suggest a genetically based trade-off between maternal effects on viability vs. growth rate. Maternal effects are often viewed in terms of investment in offspring quality and its trade-off with offspring number or parent survival. However, some physiological manipulation experiments on birds show that maternal effects on different aspects of offspring performance may trade-off with each other (e.g. Groothuis *et al.*, 2005; Schlotz *et al.*, 2013). In our study, if we quantify maternal effects as the difference between the reciprocal crosses in mean trait estimates for female offspring, the effects on growth rate and viability are negatively correlated across the population pairs (Fig. 2; Pearson's $r = -0.89$, $P = 0.018$; Spearman's $r_s = -0.94$, $P = 0.005$). Although this post hoc correlative analysis must be treated with caution, it suggests that the contribution of maternal effects to adaptation to larval malnutrition is constrained by negative genetic correlation between maternal effects on larval growth and viability. A trade-off between growth rate and viability is not apparent in our selected populations, whose adaptation to the malnutrition regime is manifested in both faster growth and higher viability (Kolss *et al.*, 2009). This implies that much of their response has been mediated

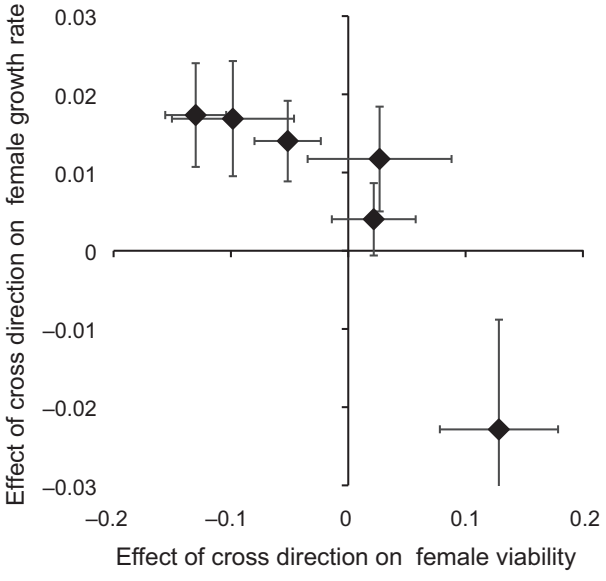


**Fig. 2** The relationship between the maternal effect of Selected mothers on growth rate and viability of daughters, estimated as the difference between the reciprocal crosses. Error bars indicate standard errors.

by alleles that improve both these fitness components, or improve one of them with no effect on the other. This, however, does not preclude a contribution of some polymorphisms with antagonistically pleiotropic effects on these two traits. In such a case, trade-offs in genetic fitness components may become apparent after many generations of adaptation to a novel environment, when genetic variation that does not show antagonistic pleiotropy has been exhausted (Archer *et al.*, 2003). A physiologically mediated trade-off between fast growth and viability has been demonstrated in larvae of several insects (Gotthard *et al.*, 1994; Blanckenhorn, 1998; Teuschl *et al.*, 2007). Our results suggest that this trade-off may be maternally mediated and affect adaptation to nutritional stress.

The above conclusions are subject to several caveats. First, the differences between reciprocal crosses that we interpreted as maternal effects could in principle also reflect the effects of cytoplasmic maternally inherited genomes, that is those of mitochondria and the endosymbiotic microbes such as *Wolbachia*. Mitochondrial genome variation can affect life history traits and their response to diet in *Drosophila* (Zhu *et al.*, 2014) and other insects (e.g. Kazancioglu & Arnqvist, 2014). There is also evidence that the presence of *Wolbachia* may affect life history in *Drosophila* (Fry *et al.*, 2004), and that the frequency of different *Wolbachia* strains may respond to selection imposed by thermal stress (Versace *et al.*, 2014), suggesting that genetic variation in *Wolbachia* may affect host stress tolerance. Our selected and control populations all carry *Wolbachia* (B. Erkosar, unpublished data). However, the complex line-cross design applied in an earlier study to population pair 1, which allowed to separate maternal form cytoplasmic effects, found little evidence for the latter. The only trait for which the most parsimonious model of genetic model included cytoplasmic effects was egg-to-adult viability, and there the estimated effect was small, about 1/4 of the maternal effect on that trait (Vijendravarma & Kawecki, 2013). The maternal effects found in that study correspond very well to those inferred from the reciprocal crosses between population pair 1 in the present study. However, the possibility remains that the evolution of cytoplasmic genomes contributed significantly to adaptation of some of selected populations.

The second caveat involves our interpretation of cross direction effects as owing to maternal rather than paternal effects. Although *Drosophila* fathers do not provision the offspring in any way, they could still affect the offspring phenotypes indirectly, through their effects on maternal physiology, for example mediated by accessory gland proteins (Crean & Bonduriansky, 2014). Such paternal effects on offspring phenotype have been found in some arthropods (Kotiaho *et al.*, 2003; Bonduriansky & Head, 2007; Buzatto *et al.*, 2012); in one dipteran species offspring phenotype may even be affected by the diet of the previous male the mother mated with (Crean *et al.*, 2014). Effects of paternal diet on offspring developmental rate have also been reported in *Drosophila* (although possibly confounded with effects on the latency to mate; Valtonen *et al.*, 2012). However, to our knowledge, no conclusive evidence exists for natural genetic variation affecting offspring performance through paternal effects in *Drosophila* (although manipulations male–female interactions may influence offspring performance; Priest *et al.*, 2008; Dowling *et al.*, 2014). Furthermore, another study demonstrated that variation attributed to parental effects in offspring viability and developmental rate under the malnutrition regime employed in the present study was mostly or entirely owing to maternal rather than paternal effects (Nepoux *et al.*, 2015). Thus, although some contribution of paternal effects cannot be excluded, it is unlikely that the parental effects we found in this study could be largely driven by paternal rather than maternal effects.

Finally, the design of the cross in principle does not allow us to distinguish the consequences of evolution of the selected vs. control populations, so our interpretation of them being the result of the evolution of the former rather than the latter relies on other arguments. The base population should have been well adapted to the laboratory conditions, including the standard food on which the control populations were subsequently maintained (Kolss *et al.*, 2009). It is thus uncontroversial to attribute the systematic differences in performance under the malnutrition conditions between the control and selected populations to adaptive evolution of the latter. The effects on male growth and developmental rate attributable to chromosome X fit this pattern in being replicated across populations and in the predicted direction. However, there is still the possibility that some of the idiosyncratic maternal effects we found could result from genetic drift in the control populations. We think this is unlikely because of both the large magnitude and ubiquity of these maternal effects. If genetic drift had generated so much variation among the control populations in maternal effects, it would have been expected to generate similar amounts of variation owing to genes that directly affect the phenotype of their carriers. Yet, we do not see much variation among the control populations in their performance under the malnutrition regime (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2011, 2012). Therefore, it is more parsimonious to attribute the idiosyncratic effects of cross direction to the evolution of maternal effects in the selected rather than control populations.

These caveats notwithstanding, our results show that evolutionary change in maternal effects can contribute substantially to short-term responses to selection imposed by nutritional stress. However, they appear to be constrained from evolving in a way that would simultaneously improve offspring growth and viability. Rather, despite uniform selection and the same initial

gene pool, they follow disparate evolutionary trajectories in different populations along an apparent trade-off between effects on offspring growth and viability. The fact that variation in life history traits among the replicate malnutrition-adapted populations is rather small (Kolss *et al.*, 2009; Vijendravarma *et al.*, 2012), despite the large variation in maternal effects found here, suggests that maternal effects and the effects of genes expressed in offspring may have co-adapted such that they compensate each other (Lancaster *et al.*, 2010; Hoyle & Ezard, 2012).

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