

## Epistasis and maternal effects in experimental adaptation to chronic nutritional stress in *Drosophila*

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**Abstract.** Based on ecological and metabolic arguments some authors predict that adaptation to novel, harsh environments should involve alleles showing negative (diminishing return) epistasis and/or that it should be mediated in part by evolution of maternal effects. While the first prediction has been supported in microbes, there has been little experimental support for either prediction in multicellular eukaryotes. Here we use a line-cross design to study the genetic architecture of adaptation to chronic larval malnutrition in a population of *Drosophila melanogaster* which evolved on an extremely nutrient-poor larval food for 84 generations. We assayed three fitness-related traits (developmental rate, adult female weight and egg-to-adult viability) under the malnutrition conditions in 14 crosses between this selected population and a non-adapted control population originally derived from the same base population. All traits showed a pattern of negative epistasis between alleles improving performance under malnutrition. Furthermore, evolutionary changes in maternal traits accounted for half of the 68 % increase in viability and for the whole of 8 % reduction in adult female body weight in the selected population (relative to unselected controls). These results thus support both of the above predictions and point to the importance of non-additive effects in adaptive microevolution.

**Keywords:** genetic architecture, experimental evolution, malnutrition, epistasis, epigenetics, line-cross analysis

### Introduction

Although the immediate response of quantitative traits to natural selection depends on additive genetic (co)variances, the long term response is predicted to be affected by non-additive gene effects, in particular epistatic interactions. Sign epistasis underlies the notion of "alternative adaptive peaks" (Whitlock et al., 1995; Weinreich et al., 2005; de Visser et al., 2011). Even without changing the effect sign, negative (diminishing-return) epistasis between favored alleles is predicted to slow down the response to selection and ultimately to limit the height of "adaptive peaks". In turn, positive (synergistic) epistasis between favored alleles increases evolvability (Whitlock et al., 1993; Keightley, 1996; Wade, 2002; Carter et al., 2005; Pigliucci, 2008; Chou et al., 2011). Epistasis may also contribute to the maintenance of genetic variation (Gimelfarb, 1989; Carlborg et al., 2006), inbreeding depression (Lynch & Walsh, 1998; Vergeer et al., 2012) and contingency of evolution (Teotonio & Rose, 2001; Schaper et al., 2012), as well as playing a crucial role in postzygotic reproductive isolation (e.g., Turelli & Orr, 2000).

Analyses of macroevolutionary patterns of gene and protein sequences suggest frequent synergistic sign epistasis between derived alleles - positive selection for more recently fixed alleles is often contingent on previous substitutions at other loci (Bridgham et al., 2009; Breen et al., 2012). Nonetheless, this pattern may largely reflect interactions between alleles fixed millions of generation

apart, in response to different factors of natural selection acting on different traits; the interacting loci may never have been simultaneously polymorphic (Whitlock et al., 1995).

In contrast, to what degree adaptive microevolution is influenced by interactions between polymorphisms affecting the same trait and/or subject to the same force of selection remains controversial. In particular, some authors (Hill et al., 2008; Crow, 2010) argue that epistatic variance has negligible influence on the evolution of ecologically relevant quantitative traits. This opinion might seem inconsistent with the pervasive contribution of epistasis to standing genetic variance for such traits (Kelly, 2005; Roff & Emerson, 2006; Huang et al., 2012). Nonetheless, the architecture of standing genetic variation does not necessarily predict the response to directional selection beyond several generations, in particular if the response transgresses the original range of genotypic variation (Hill & Mbagwa, 1998; Stepan et al., 2002). Similarly, epistasis between deleterious mutations throws little light on the interaction between mutations that would mediate adaptation (de Visser et al., 2011). Some evidence for the importance of epistasis for local adaptation comes from the analysis of natural populations. E.g., following colonization of novel habitats, the evolution of photoperiod response in the pitcher-plant mosquito (Hard et al., 1993) and of coat color in the old field mouse (Steiner et al., 2007) involved synergistic (positive) epistasis between derived alleles. However, for much of the large literature on genetic architecture of divergence between natural populations (e.g., Gilchrist & Partridge, 1999; Fenster & Galloway, 2000; Demuth & Wade, 2007; Wegner et al., 2008; Kennington & Hoffmann, 2010; Pritchard et al., 2013) the ancestral state and the selection forces driving the divergence remain poorly known. Furthermore, genetic architecture is often sensitive to the environmental conditions during assay (Blows & Sokolowski, 1995; Demuth & Wade, 2007; van Heerwaarden & Sgro, 2011; Bublly et al., 2012). Thus, epistatic effects in common garden crosses between different natural gene pools do not necessarily imply epistasis within a gene pool between alleles that mediate adaptation of each population to its environment.

Such ambiguities can be minimized by using experimental evolution: allowing experimental populations to evolve in a controlled environment and then investigating the genetic architecture of their divergence from the ancestor (or from an appropriate control population) within the same environment. This approach has supported the importance of epistasis in adaptive evolution of microbes, where mutations conferring incremental improvements in fitness rather consistently show negative epistasis (de Visser et al., 1999; Bull et al., 2003; MacLean et al., 2010; Chou et al., 2011; Khan et al., 2011; Wang et al., 2013) whereas the evolution of a major metabolic novelty involved synergistic (sign) epistasis (Blount et al., 2012). Of studies addressing the genetic architecture of response to artificial selection on more or less arbitrary characters in plants and animals, in some the response appears to be essentially additive among loci (e.g., Laurie et al., 2004) while others found a substantial contribution of epistasis (e.g., Carlborg et al., 2006) (for older examples see Lynch and Walsh 1998, Table 9.5). However, evolution under strong artificial selection applied to a single character while minimizing other differences in fitness may be less representative of evolution in nature than experimental evolution driven by natural selection, even under controlled laboratory conditions (Hill, 2011; Kawecki et al., 2012). Tests of epistasis under such "laboratory natural selection" in multicellular eukaryotes are scarce. Teotonio et al. (2004) found little contribution of epistasis to the evolution of fitness traits in *Drosophila* populations adapted to periodic adult starvation or to a short generation regime. Likewise, adaptation of *Arabidopsis thaliana* to crowding (Ungerer et al., 2003; Ungerer & Rieseberg, 2003) and a seed beetle to two novel host species (Fox et al., 2011; Messina & Jones, 2011) has largely been based on alleles with additive effects. Only one of several traits studied (hatching success) showed some evidence of epistasis contributing to differentiation between populations of the seed beetle after 150 generations of convergent evolution in the same environment (Bieri & Kawecki, 2003). Thus, in contrast to microbes, evolution experiments in plants

and animals so far offer little support for the importance of epistasis in adaptation to novel environments.

Evolution experiments can also contribute to the understanding of the evolutionary significance of maternal effects, defined as the causal influence of differences in maternal phenotype on offspring phenotype (Mousseau & Fox, 1998; Wolf & Wade, 2009). While much attention has been devoted to adaptive maternal effects induced by the maternal environment (maternally-mediated plasticity, Mousseau & Fox, 1998; Agrawal et al., 1999; Duckworth, 2009), it is less clear how genetic evolution of maternal effects contributes to adaptation to novel environments (Badyaev & Uller, 2009). This requires genetic variation for maternal effects, whereby offspring phenotype is causally affected by the maternal genotype (other than by having inherited maternal genes). Such "genetic maternal effects" (Falconer & Mackay, 1996; Lynch & Walsh, 1998) contribute a substantial fraction of phenotypic variance in a variety of traits and species under both natural and laboratory conditions (reviewed by Rasanen & Kruuk, 2007). Theory predicts that evolution of maternal effects may accelerate the response of offspring traits to selection (Kirkpatrick & Lande, 1989). This may be particularly important in novel, stressful environments where juvenile survival and early development may be more affected by the genetic variation in the amount of provisioning or "priming" of the offspring by the mothers than by the juveniles' own genomes (Badyaev & Uller, 2009). Such "positive" or "synergistic" maternal effects have contributed e.g. to adaptation of moor frog populations to acidification (Hangartner et al., 2012) or to rapid evolution of male morphological traits in an invasive population of the house finch (Badyaev, 2005). They also often contribute to artificial selection response for juvenile growth or size in various species (e.g., Goodwill, 1975; Rhees et al., 1999; Park et al., 2006). However, due to genetic correlations with other traits under selection, maternal effects may also evolve in a direction opposite to that favored by selection on the maternally affected traits. Such "antagonistic" maternal effects are expected to introduce a lag in the evolutionary response or even lead to responses in opposite direction to that favored by selection (Kirkpatrick & Lande, 1989). In two evolution experiments, antagonistic maternal effects partially counteracted adaptation to periodic food absence and short generation time in *D. melanogaster*, in particular by exacerbating apparent trade-offs; a positive contribution of maternal effects to adaptation was only found for one trait in one sex in one of those experiments (Teotonio et al., 2004). Thus, maternal effects do sometimes impede rather than promote adaptation to novel environments, but how common this is remains unclear; several other studies found no maternal effects on experimentally evolved divergence in fitness-relevant traits (Hutchinson et al., 1991; Fox et al., 2011; Messina & Jones, 2011).

In this paper we address the contribution of epistatic and maternal effects to the genetic architecture of experimental adaptation to chronic larval malnutrition in *Drosophila melanogaster*. Specifically, we study the architecture of divergence between a population selected for malnutrition tolerance for 84 generations and a control population. These two populations originate from a replicated experimental evolution study in which six selected populations have been maintained on an extremely nutrient-poor larval food while six control populations have been maintained on standard food. The quality of the poor food is such that non-adapted flies have poor survival, need twice as long time for larval development and still emerge at half the adult weight of flies raised on the standard food (Kolss et al., 2009). The selected populations have evolved markedly improved tolerance to this form of chronic malnutrition, manifested as higher egg-to-adult viability, faster growth, shorter development, and a smaller critical size for successful pupation (Kolss et al., 2009; Vijendravarma et al., 2012b). They also show improved larval competitive ability, a more 'sitter' like foraging behaviour and a greater propensity to cannibalism (Vijendravarma et al., 2012c; Vijendravarma et al., 2012a; Vijendravarma et al., 2013), pointing to involvement of many traits in this experimental adaptation.

We performed a two-generation line-cross analysis (Lynch & Walsh, 1998) to estimate composite genetic effects contributing to the divergence between the selected and the control population in three fitness-related traits (developmental rate, body weight at emergence and egg-to-adult viability) expressed under the malnutrition conditions (i.e., on the poor food). While it does not lead to identification of individual genes, this approach reveals overall ("average") patterns of genetic architecture underlying phenotypic divergence between populations (Lynch & Walsh, 1998, Chapter 9), in this case pointing to pervasive epistasis and maternal effects.

## Materials and methods

### *Origin and maintenance of fly populations*

We use the first replicate population subject to a malnutrition selection regime (S1) and the first control population (C1); as the replicate numbers were arbitrarily assigned before the commencement of selection, they are effectively random representatives of each regime. Their origin and the experimental evolution regimes are detailed elsewhere (Kolss et al., 2009; Vijendravarma et al., 2012b). Briefly, the selected population was maintained for 84 generations on a poor food, containing 12.5 g cornmeal, 7.5 g sucrose, 15 g glucose, 3.2 g dry yeast, 0.5 g MgSO<sub>4</sub>, 0.5 g CaCl<sub>2</sub>, 30 ml ethanol, 6 ml propionic acid and 1 g nipagin per litre of water. This corresponds to ¼ of the nutritional content of standard food, on which the control population was maintained. The larval densities were regulated at 200 to 250 larvae per 30ml of food. At this density the energy and protein content of the poor food (at least 300 J and 1.2 mg of protein per larva) should in theory be more than sufficient to support the development of all larvae - a fully grown larva under optimal conditions contains about 11.5 J and 0.13 mg of protein. Thus, competition was low and the main challenge for the larvae was the low concentration of nutrients and presumably gradual contamination of the food source with metabolic waste products. Adults of both selection regimes were maintained on standard food with a live yeast supplement, i.e., the nutritional stress was limited to the larval stage. To eliminate any effects due to parental environment, both populations (C1 and S1) were reared for two generations at a controlled density on standard food prior to the assays. All assays were performed at 25 °C and 70 % humidity.

### *Line-cross design*

We studied the phenotypes of 14 crosses derived by crossing the control population with the selected population over two generations. Following the generic notation used in line-cross analyses, in the rest of the paper we refer to those populations as parental lines P<sub>1</sub> and P<sub>2</sub>, respectively. The crossing scheme followed that implemented by Bieri and Kawecki (2003), the crosses are defined in the first two columns of Table 1. To obtain parents of the individuals to be assayed, crosses between males and virgin females of the parental lines P<sub>1</sub> and P<sub>2</sub> (in both directions) were set up to generate F<sub>1</sub> and F<sub>1R</sub> hybrid parents; matings between males and virgin females within the parental lines generated P<sub>1</sub> and P<sub>2</sub> parents. These parents were raised in multiple bottles on standard food; virgin females and males were collected upon emergence and maintained on standard food with live yeast for 5-6 days. They were then used to set up the 14 crosses. Thus the phenotypes of all the crosses (including the parental lines) were assayed simultaneously and were the first generation to be raised on poor food since the relaxation of selection three generations earlier. For each cross four replicate bottles containing 30 ml of poor food were set up and were each seeded with exactly 200 eggs; each replicate bottle originated from a mass-mating between a separate set of 20-30 parents of each sex. The number of emerging adults in each bottle and their sex was recorded once every 24 h. Twelve newly eclosed females were

collected from each bottle on the day of peak emergence (or two days if necessary) and dried at 70 °C in an oven for three days and weighed as a group to the nearest microgram.

### *Analysis*

*Cross means and standard errors.* For each surviving individual we calculated the developmental rate as the inverse of the developmental time (in days). The mean developmental rate was then calculated separately for males and females for each replicate vial. Viability was estimated for each replicate vial as the number of eclosed adults divided by the number of eggs. Because the sex of individuals that failed to survive was unknown, we could not directly assay sex-specific viability. Nonetheless, we estimated sex-specific viability values assuming that 50 % of eggs in each vial were of each sex. This latter approach allowed us to fit models including the effects of X chromosome (see below). However, the assumption of 50:50 primary sex ratio remains untested, so we also report the analysis based on the pooled viability of both sexes. The average female dry weight at eclosion for each replicate vial was estimated from the pooled weight of 12 females. The vial means for each trait were used to estimate the overall mean and its standard error for each of the 14 lines.

*Model fitting.* We used the mean phenotypic values of the 14 crosses and their standard errors to fit linear models of genetic architecture, using weighted least-square regression as described by (Lynch & Walsh, 1998, Chapter 9). The composite genetic parameters and their regression coefficients are listed in Table 1. The composite additive [ $a$ ], dominance [ $d$ ], and epistatic terms [ $a \times a$ ], [ $a \times d$ ] and [ $d \times d$ ] are defined as in Lynch and Walsh (1998, Chapter 9). The additive and dominance maternal effects [ $am$ ] and [ $dm$ ] denote the effects of maternal genotype on offspring, so the regression coefficients are the same as the regression coefficients for [ $a$ ] and [ $d$ ] of their maternal lines. Because the X chromosome contains about 20 % of the *Drosophila* genome, we also included two parameters, [ $Xa$ ] and [ $Xd$ ], to describe the composite additive and dominance effects of the X chromosome in female flies, and a single parameter [ $Xh$ ] corresponding to the haploid effect of the X chromosome in males. The last parameter, [ $c$ ], reflects the contribution of cytoplasmic factors (in particular the mitochondrial genome).

Models with X-chromosome effects were fitted either to data from a single sex (dry weight, which was only measured for females), or jointly to both sexes (developmental time, viability). In the latter case, the data consisted of 28-element vectors (14 lines  $\times$  2 sexes). In those models the regression coefficients for [ $Xa$ ] and [ $Xd$ ] were set to zero for males, while those for [ $Xh$ ] were set to zero for females; the other coefficients were the same for both sexes. Additionally, a parameter  $s$  corresponding to the mean difference between sexes was included in these models, with coefficient equal 0 for females and 1 for males. It can be noted that in our crossing scheme the coefficients for the predicted contributions of the X chromosome to male phenotype were identical to those for additive maternal effects (the columns for [ $ma$ ] and [ $Xh$ ] in Table 1 are identical). Thus the contributions of those two composite effects cannot be distinguished in models fitted to male data only; however, they can still be simultaneously fitted to data on both sexes, because the additive maternal effects are assumed to contribute to the phenotype of both sexes whereas the haploid effect of the X chromosome only contributes to the male phenotype.

*Model selection.* To decide which set of composite genetic parameters best explains the pattern of cross means we used the Akaike Information Criterion (AIC), followed by significance testing of individual parameters included in the selected model. The model for which the AIC is minimized is deemed the most parsimonious, i.e., striking an optimal balance between the amount of variation explained and the number of parameters in the model (Burnham & Anderson, 1998). This approach is preferred over stepwise regression in cases where, as here, the number of potential parameters is large and the models compared do not form a clearly hierarchical structure (Burnham & Anderson, 1998).

In the present case the AIC is given by the weighted residual sum of squares plus twice the number of model parameters plus a constant (Bieri & Kawecki, 2003). With  $n$  parameters that could be either included or excluded, the number of possible models is  $2^n$ . While in principle all these models might be compared for the AIC, comparison of such large number of models is not desirable on statistical grounds (Burnham & Anderson, 1998). To reduce the number of models to be compared, we followed Bieri and Kawecki (2003): we grouped the three epistatic parameters, the two maternal effects parameters and the three X chromosome parameters, and only compared models that either included or excluded all parameters of a given group. E.g., a model with all three epistatic parameters was compared for AIC to one assuming no epistasis, but a model only including additive-additive epistasis was not considered. This is justified by noting that the definitions of the different types of epistatic interactions depend in part on the frame of reference; e.g., they differ between the parameterization assumed here (following Lynch & Walsh, 1998) and one proposed by Kearsley and Pooni (1996). We were thus more interested in the overall contribution of epistasis, maternal effects and X-chromosome effects than in their subdivision into components.

Under an additive-dominance model the mean of  $F_2$  crosses should correspond to the midpoint between the midparent and the  $F_1$  mean, i.e.,  $(P_1 + P_2)/4 + F_1/2$ . The scaling parameter  $C = 4F_2 - 2F_1 - P_1 - P_2$  (where  $F_1$  and  $F_2$  refers to the averages of the respective reciprocal crosses) is often used to quantify deviations from this model (Mather & Jinks, 1982). However,  $C$  is affected by both epistatic and dominance maternal effects (under our parameterization the expected value of  $C$  equals  $-2[a \times a] - 4[d \times d] + 8[dm]$ ). To isolate the contribution of epistatic effects to the performance of  $F_2$  relative to the additive-dominance expectation we defined parameter  $C_e = -(1/2)[a \times a] - [d \times d]$ . A positive (negative) value of  $C_e$  implies that epistatic effects increase (reduce) the performance of  $F_2$  crosses relative to what would be expected in the absence of epistasis, and the value corresponds directly to the magnitude of the effect. The standard error of  $C_e$  was estimated from the error covariance matrix of  $[a \times a]$  and  $[d \times d]$ .

*Testing the significance of parameters.* After the most parsimonious model was selected, we used the likelihood ratio test to test the significance of each parameter included in this model. We also tested the joint significance of the three epistatic parameters whether or not they were included in the most parsimonious model (Bieri & Kawecki, 2003). Analogous approach was used to test the joint significance of the X chromosome effects and the maternal effects. Finally, we tested the overall fit of the model as described in Lynch and Walsh (1998, p. 217).

*Exploratory analysis of other composite interaction parameters.* Where the most parsimonious of the a priori models did not provide a good fit to the data, we explored how additional parameters representing interactions between effects (e.g., between cytoplasmic and autosomal or X-chromosome effects) might improve the fit. The regression coefficients for those parameters were products of the coefficients for the main effects contributing to the interaction (Lynch & Walsh, 1998). The candidate interactions were chosen based on inspection of the pattern of cross means and their genetic composition.

## Results

The selected line ( $P_2$ ) developed 22 % faster ( $t_6 = 5.98$ ,  $P = 0.0009$ ) and survived 68 % better ( $t_6 = 4.5$ ,  $P = 0.004$ ) to adulthood than the control line ( $P_1$ ), but showed an 8 % lower mean female adult body weight ( $t_6 = 3.6$ ,  $P = 0.01$ ). This confirms previous results reported in Kolss et al. (2009).

The pattern of crosses' mean phenotypes was similar for all traits (Fig. 1). The first generation crosses in either direction showed no evidence of heterosis. For developmental rate and viability the mean phenotypes of both  $F_1$  crosses were almost exactly intermediate between the two parental lines.

The same held for the female weight of the  $F_1$  cross, whereas the reciprocal  $F_{1R}$  cross had a lower weight than the midparent, and significantly lower than the  $F_1$  cross ( $t_6 = 2.5$ ,  $P = 0.048$ , although this difference would not remain significant after correction for multiple comparisons).

In contrast to the first-generation crosses, for most (viability and female weight) or all (developmental rate) second-generation crosses the mean phenotypes exceeded the values predicted by a simple additive model (line linking the two parental means in Fig. 1). This was particularly striking for female weight, where seven out of eight backcrosses were heavier on average than the heavier parental line. Thus, an additive model was clearly inadequate to explain the pattern of second-generation crosses. The most parsimonious models for all traits (Table 2) included a large contribution of epistasis, as well as X-chromosome, maternal or cytoplasmic effects reflecting differences between reciprocal crosses. Below we describe the results for each trait in detail.

*Developmental rate.* The most parsimonious models for developmental rate excluded the dominance parameter [ $d$ ], but included a large negative dominance  $\times$  dominance epistatic effect [ $d \times d$ ]; this is the epistatic parameter reflecting the difference between  $F_2$  and  $F_1$  crosses (cf. Table 1). The smaller positive additive  $\times$  additive epistatic [ $a \times d$ ] parameter is likely accounted for by the tendency of backcrosses to do somewhat better than the  $F_2$  and  $F_{2R}$  flies and the first generation crosses to do somewhat less well than the average of the parental lines, after X-chromosome effects have been accounted for. Overall, the contribution of epistasis to the deviation of second generation means from the additive expectation (as quantified by the parameter  $C_e$ ) equaled about 1/3 of the divergence between the parental lines. The additive composite parameter [ $a$ ] accounted for about 2/3 of the difference between the means of the two parental lines ( $2 \times [a] \approx 0.14$ ). The rest of the difference was attributed to additive  $\times$  dominance epistasis in both sexes, additive effects of the X chromosome in females (both not significant but retained in the most parsimonious model), and in particular to the effects of X chromosome in males [ $Xh$ ]. The haploid effect of the X chromosome in males accounts for the difference between  $F_1$  and  $F_{1R}$  crosses apparent for males but not females.

The analysis did not detect maternal or cytoplasmic effects on developmental rate. Males developed slightly faster than females (as indicated by the positive value of parameter  $s$ ), but, except for the difference between  $F_1$  and  $F_{1R}$ , the pattern of means was nearly identical for the two sexes (Fig. 1a,b; with  $F_1$  and  $F_{1R}$  excluded, Pearson's correlation of male and female means  $r > 0.99$ ). Overall, the most parsimonious model for developmental rate explained 91 % of variation among line means (weighted by their sampling variances; Lynch & Walsh, 1998), a rather unsatisfactory fit. Including the dominance, maternal and cytoplasmic effects (omitted from the most parsimonious model) had negligible effect on the goodness-to-fit (details not shown).

We therefore explored how including some additional parameters representing interactions between composite effects affects the fit of the model. The main reason for the poor fit were the differences between reciprocal backcrosses, which the most parsimonious model predicted to be similar or identical (in particular, it predicted  $B_{1Ra} = B_{1Rb}$  and  $B_{2Ra} = B_{2Rb}$  for both sexes, which did not seem to be the case for the observed means). We first considered epistatic interactions between the autosomal and X-chromosome effects, but only the interaction between autosomal additive and X dominance effects was marginally significant ( $P = 0.05$ ). Because the X dominance effect is only defined for females, this interaction could only improve the fit of the female means, and the overall model fit remained poor. However, we noticed that two backcrosses,  $B_{1Rb}$  and  $B_{2Ra}$ , which in both sexes tended to be above the other backcrosses, are characterized by having the cytoplasm from the control parental line  $P_1$  and 3/4 of the autosomal genes from the selected parental line  $P_2$  (cf. Table 1). Thus, even though cytoplasmic effects were excluded from the most parsimonious model, we now included them and their interaction with autosomal and X-chromosome effects. This analysis suggested an antagonistic interaction between the cytoplasmic and autosomal effects (composite parameter [ $a \times c$ ] =

$-0.063 \pm 0.010$ ,  $\chi^2 = 37.4$ ,  $df = 1$ ,  $P < 0.0001$ ), to some degree compensated in males by a synergistic interaction between the cytoplasmic and X-chromosome effects (composite parameter  $[Xh \times c] = 0.022 \pm 0.007$ ,  $\chi^2 = 10.4$ ,  $df = 1$ ,  $P = 0.0012$ ); the main cytoplasmic effect was not significant ( $[c] = -0.004 \pm 0.004$ ,  $\chi^2 = 1.6$ ,  $df = 1$ ,  $P = 0.21$ ). A model with these additional parameters had a considerably better fit than the most parsimonious of the a priori models ( $R^2 = 0.97$ ,  $\chi^2 = 23.3$ ,  $df = 16$ ,  $P = 0.11$ ). This post-hoc analysis should be treated with caution, possibly inclusion of other interaction parameters (including 3-way interactions and interactions involving sex) would result in similarly good fit. Importantly, including these additional parameters did not change the conclusions from the most parsimonious a priori model about the contribution of additive, epistatic and X-chromosome effects. Although the parameter values changed somewhat, those that were significant ( $[a]$ ,  $[a \times a]$ ,  $[d \times d]$ ,  $[Xh]$ ) remained so, and none of the others became significant (details not shown).

*Body weight.* As for developmental rate, the most parsimonious model for female body weight (Table 1) excluded dominance but included epistasis, in particular dominance  $\times$  dominance epistasis, to account for the backcrosses being heavier than expected based on the  $F_1$  and parental lines (as quantified by the  $C_e$  scaling parameter). The model attributed the 8 % decline in the selected line ( $P_2$ ) compared to the control line ( $P_1$ ) mostly to additive maternal effects. The predicted difference between  $P_2$  and  $P_1$  is  $2([a] - [a \times d] + [Xa] + [c] + [am])$ ; thus, the contribution of X chromosome to this difference was cancelled by the  $[a \times d]$  epistatic parameter. The composite additive parameter  $[a]$  has been left out of the MP model; if included in the model, this parameter was far from significant ( $P = 0.48$ ) and its estimate was positive, in contrast to the sign of difference between  $P_2$  and  $P_1$ . The additive maternal effects accounted for the difference between the reciprocal first generation crosses (Fig. 1c). This last difference might also be explained by cytoplasmic effects, but this effect should then also have resulted in  $F_2$  being heavier than  $F_{2R}$  whereas the opposite trend was observed. The cytoplasmic effects were thus excluded from the model. The additive and dominance maternal effects taken together meant that non-genetically transmitted influence of the maternal genotype made offspring of  $P_2$  smaller than the offspring of  $P_1$ ,  $F_1$  and  $F_{1R}$  mothers, while the three latter lines were roughly equivalent in that respect. Overall, with  $R^2 = 0.99$  the model had a good fit to the data.

A striking feature of the line-cross means of female weight was the difference between  $B_{2b}$  and other backcrosses (Fig. 1c). As the selected parental line  $P_2$ , but in contrast to all other crosses,  $B_{2b}$  carried two X chromosomes from the  $P_2$  parental line. The model thus attributed most of this difference to the homozygous effects of X chromosome originating from the parental line  $P_2$ , combined with maternal effects (it could not be explained by autosomal effects as they do not contribute to differences between backcrosses to the same parental line, cf Table 1). Because of its idiosyncratic behavior and small sampling variance,  $B_{2b}$  was likely to have a disproportional effect on the parameter estimates. To see how robust the model was, we re-run the analysis with  $B_{2b}$  removed. The most parsimonious model for this reduced data set had a good fit ( $R^2 = 0.96$ ,  $\chi^2 = 5.2$ ,  $df = 6$ ,  $P = 0.51$ ). Compared to the most parsimonious model for the full data set, this new model included a marginally significant dominance parameter ( $[d] = 0.10 \pm 0.06$ ,  $\chi^2 = 3.4$ ,  $df = 1$ ,  $P = 0.064$ ), but excluded X-chromosome effects. Furthermore, the composite parameter for dominance maternal effects was not significant any more ( $P = 0.39$ ). The parameters  $[a \times a]$ ,  $[d \times d]$  and  $[am]$  remained significant and similar to those in the model fitted to complete data. Thus, the conclusion about epistasis and additive maternal effects were robust to exclusion of the  $B_{2b}$  cross from the analysis.

*Egg-to-adult viability.* We analyzed both the unsexed viability (Fig. 1d), as well as the sex-specific viability estimates based on the assumption of a 50:50 primary sex ratio (Fig. 1 e,f). As indicated by the parameter  $[s]$  in the most parsimonious model fitted to the sex-specific data (Table 2), males had on average lower viability (or, alternatively, the primary sex ratio was female biased). Male and female viability mean estimates showed a roughly similar pattern, but were far from perfectly

correlated ( $r = 0.85$ ). Nonetheless, the differences between female and male viability estimates were inconsistent with a significant contribution of the X chromosome effects, which were excluded from the most parsimonious model for the sex-specific analysis (models with X-chromosome effects could not be fitted to unsexed data). Both unsexed and sex-specific analyses indicated negative dominance and strong epistatic effects; the scaling parameter  $C_e$  roughly corresponded to half of the divergence between parental line. A large additive maternal effect parameter [ $ma$ ] indicates that offspring of  $P_2$  mothers had an extra advantage not mediated by the offspring genotype. The most parsimonious sex-specific model omitted the autosomal additive parameter [ $a$ ] (which was included in the most parsimonious unsexed model), explaining the difference between the parental lines in terms of additive  $\times$  dominance epistasis and additive maternal effects. The cytoplasmic effect also included in both models was negative, meaning that cytoplasm from the selected line  $P_2$  had a small negative effect on viability. With only about 90 % of variation among lines/crosses explained, the fit of either model was not great.

One potential reason for the poor fit could be due to the magnitude of the effects being sex dependent. In particular, the difference between the two parental lines tended to be greater for males than females (Fig. 1e,f; parental line  $\times$  sex  $F_{1,12} = 3.7$ ,  $P = 0.08$ ). We therefore standardized the data for each sex by first subtracting the sex-specific midparent value and then dividing by the difference between the parental means. The most parsimonious model for these standardized data had a slightly better fit ( $R^2 = 0.91$ ,  $\chi^2 = 27.5$ ,  $df = 19$ ,  $P = 0.09$ ) and was similar (in terms of parameter values and significance) to the model fitted to non-standardized sex-specific data (details not shown).

## Discussion

### *Negative epistasis between favored alleles*

The line-cross revealed a pattern of epistatic interactions that pushed the trait means of second generation crosses above the values predicted by the additive-dominance model. This pattern was similar for all three traits, although at first sight it was not consistent with respect to the direction of evolution under malnutrition – the selected population showed faster development and higher survival, but lower weight than the control.

Interpretation of these results is aided by considering the regulation of development in holometabolous insects. According to the prevailing model (Edgar, 2006), larvae continue to grow until reaching a critical size, at which physiological processes leading to pupation are initiated. From that moment, the larvae are committed to pupation and the time to pupation is not affected by nutritional conditions, even though the larvae continue to feed and grow for some time. The selected populations evolved faster growth rate on the poor food and a smaller critical size for pupation initiation (Kolss et al., 2009; Vijendravarma et al., 2012b); the former presumably reflects their greater efficiency in extracting nutrients from the poor food, while the latter enables them to complete development with less total resources accumulated. Both faster growth and smaller critical size lead to faster development by shortening the time needed to reach the critical size. In contrast, their effects on adult size are opposite – lower critical size reduces adult size while faster (post-critical) growth increases it; the slightly smaller weight of the selected populations reflects the net outcome of these two opposing effects (Kolss et al., 2009; Vijendravarma et al., 2011; Vijendravarma et al., 2012b). In the present study, the fact that epistasis led to both faster development and greater female weight of  $F_2$  and backcrosses indicates that these epistatic effects on weight were largely mediated by difference in growth rather than the critical size for pupation initiation. Combining this with the viability results, and taking into account that growth rate and viability are highly inclusive indices of larval performance, one is led to conclude that epistatic interactions resulted in the larval performance of the second-generation crosses being higher than expected in the absence of epistasis.

While egg-to-adult viability has been measured on a random sample of all offspring produced, developmental rate and adult weight can only be measured on individuals that survive to adulthood. As survival may not be random with respect to alleles affecting adult traits, the estimates of the latter could be biased. Such within-generation selection is an inherent problem of any study estimating adult trait means. If gene combinations favoring survival also increased developmental rate and body weight, the means of those traits in  $F_2$  and backcrosses would be overestimated. However, this could not explain the means of those traits for some of the backcrosses exceeding both parental means. Furthermore, while selection imposed by the larval poor food regime does promote fast development, it favors small rather than large body size (Vijendravarma et al., 2011; Vijendravarma et al., 2012b). And, egg-to-adult viability estimates, which are free of this bias, show qualitatively similar patterns of epistasis. Thus, while within-generation selection may have introduced some bias in estimates of adult weight and developmental rate, they are highly unlikely to be responsible for the effects attributed by the analysis to epistatic interactions.

The conclusions about epistasis may depend on the scale (Lynch & Walsh, 1998). Because of the multiplicative relationship between egg-to-adult viability and fitness, it could be argued that, as for fitness itself, epistasis should be defined as deviation from multiplicative effects (de Visser et al., 2011), rather than from additivity as assumed in our analysis. However, a logarithmic transformation of viability data did not affect the conclusions about epistasis; in fact, the most parsimonious model for pooled log-transformed viability data included the same composite effects as that for untransformed data, with all three epistatic parameters significantly negative (details not shown). While it is not clear what the most relevant scale for developmental rate and body weight should be, it is difficult to imagine a biologically meaningful transformation under which the patterns of crosses would be consistent with an additive-dominance model, in particular for weight. Thus, the epistatic interactions we detected are unlikely to be an artifact of scale.

While new mutations are unlikely to have contributed significantly (Hermisson & Pennings, 2005), both selection and drift acting on the original standing variation have probably contributed to genetic divergence between the selected and control parental lines. Not all genetic differences contributing to the patterns of crosses must have necessarily contributed to the difference of the trait means between the parental lines. In particular, genetic drift may have led to fixation of different partially or fully recessive deleterious alleles in  $P_1$  and  $P_2$ , reducing the mean performance of both populations (inbreeding depression), rather than contributing to their phenotypic divergence. Given the moderately small population sizes of 150 breeding adults over 84 generations, inbreeding depression for our focal traits would not be unexpected (although none was detected after 29 generations, Kolss et al., 2009). Some forms of epistasis between alleles responsible for inbreeding depression may improve the performance of second-generation crosses above the additive-dominance expectation (Lynch & Walsh, 1998). However, in such a case restoration of heterozygosity should be manifested as an improvement in the performance of first-generation crosses and as a positive dominance coefficient [ $d$ ]. This was not the case; dominance was excluded from the most parsimonious model for developmental rate and female weight, and was significantly negative for viability. Thus, drift-driven accumulation of different recessive deleterious alleles seems unlikely as a major explanation for the pattern of epistasis detected in the crosses.

We conclude therefore that the deviations of the second-generation crosses from the additive-dominance expectation largely result from epistasis between alleles underlying the divergence between the parental means. Furthermore, the common pattern observed for all three traits suggests that the epistatic effects are mediated through a common physiological mechanism, possibly related to digestive and metabolic efficiency. Although only one selected and one control population were included in this study, they are representative of the respective sets of selected and control

populations; the responses of life history traits have been highly parallel across the replicates populations (Kolss et al., 2009; Vijendravarma et al., 2011; Vijendravarma et al., 2012b). This parallelism indicates that the divergence whose architecture we analyzed here has been driven by selection rather than by drift. Furthermore, the base population from which the control and selected populations were derived should have been adapted to the standard food and other aspects of the laboratory conditions (Kolss et al., 2009). Thus, the divergence between the control and selected populations in larval traits on poor food should mostly reflect the evolution of the selected population in response to the malnutrition selection regime. From this perspective, our results imply that alleles that improved larval adaptation to the poor food in the selected population show negative (diminishing return) epistasis (de Visser et al., 2011): their joint effect is smaller than the sum of individual effects. In other words, the mean larval performance of the selected population  $P_2$  is lower than would be expected by extrapolating from the mean performance of recombinants ( $F_2$  and backcrosses) carrying intermediate frequencies of those favored alleles. Such negative epistasis between beneficial alleles has been predicted by theory based on the Fisher's geometric model of adaptation (Martin et al., 2007; Gordo & Campos, 2013). Moreover, based on metabolic control theory (Szathmary, 1993; You & Yin, 2002) and ecological consideration (Crow & Kimura, 1979; Kouyos et al., 2007), several authors predict that negative epistasis should be prevalent under nutrient limitation or other harsh environments. Consistent with this theory, negative epistasis between favored alleles has been reported in several microbial evolution experiments (see the introduction) and in analyses of mutants (Martin et al., 2007). However, as summarized in the introduction, evidence for such a pattern of epistasis in short-term adaptation of sexual multicellular eukaryotes has been scarce. The negative epistasis among alleles contributing to experimental evolution of malnutrition tolerance inferred in this study thus provides a welcome support for this theoretical prediction.

The exploratory post-hoc analysis of the pattern of developmental rate additionally suggested a rather strong negative epistasis between nuclear and cytoplasmic genes. Taken at a face value, this would mean that both control and selected populations would develop faster if they swapped their cytoplasmic genomes. Nuclear  $\times$  cytoplasmic epistasis has been previously found in *D. melanogaster* and attributed to variation in compatibility between nuclear- and mitochondrially-encoded components of translation machinery (Montooth et al., 2010; Meiklejohn et al., 2013). One could speculate that such nuclear-mitochondrial interactions would be of particular importance under strong nutrient limitations imposed in our study, where energetic efficiency is likely to be under strong selection. However, because of the post-hoc nature of this analysis, the conclusion about the epistasis between cytoplasmic and nuclear genes in our study remains highly tentative. If confirmed, it would further strengthen the general notion that negative epistatic interactions hamper or constrain adaptation to nutritional stress even within a short evolutionary timescale.

#### *Maternal effects contribute to evolutionary change*

Our analysis revealed significant additive maternal effects for adult body weight and viability (dominance maternal effects for these two traits were also present, but they were small and are not discussed further). For viability, the additive maternal effects were estimated to contribute about half of the large increase in viability of the selected ( $P_2$ ) line relative to the control ( $P_1$ ) line; for body weight, the magnitude of the additive maternal effect was such that it alone could account for the lower weight of  $P_2$ . We detected no maternal effects for developmental rate; the (non-significant) parameter [*ma*] for this trait was negative when included in the model (details not shown). Therefore, the additive maternal effect on weight seems to be mediated by slower growth rather than smaller critical size, and thus probably reduces the offspring fitness. In contrast, the maternal effect on viability is rather unequivocally adaptive, and it is large – having a mother from the selected rather than control line increased the probability of survival to adulthood by 25 % relative to the midparent

value (33 % relative to the viability of the control population). This is presumably more than enough to compensate for the smaller (8 %) negative maternal effect on weight.

Our results thus support the notion that adaptation to novel environments, particularly those that are stressful to juveniles, may to a large degree be mediated by evolution of maternal traits. In that, they are consistent with the importance of maternal effects in local adaptation inferred from some studies of natural populations (Badyaev & Uller, 2009). However, as reviewed in the introduction, such adaptive maternal effects have not been commonly found in experimental evolution under stressful environments. Rather, genetic maternal effects were either not detected (Hutchinson et al., 1991; Fox et al., 2011; Messina & Jones, 2011) or were antagonistic to the direction of selection (Teotonio et al., 2004). The negative maternal effect on weight in our study is nonetheless consistent with the contribution of maternal effects to evolutionary trade-offs, analogous to those detected in *Drosophila* populations selected for starvation resistance and longevity (Teotonio et al., 2004).

Maternal effects can be mediated through provisioning eggs with more nutrients or through priming the developing embryo through e.g. hormonal signals, mRNAs or small RNAs in the cytoplasm or epigenetic chromatic modification (Mousseau & Fox, 1998; Badyaev & Uller, 2009; Wolf & Wade, 2009). While the contribution of those mechanisms to adaptive genetic maternal effects remains unclear, they have been found to mediate adaptive effects induced by maternal environment, i.e., plastic maternal effects (e.g., Perrin, 1989; Kawecki, 1995; Fox et al., 1997; Agrawal, 2002; Whittle et al., 2009; Rasmann et al., 2012). We have previously (Vijendravarma et al., 2010) reported environmental plastic maternal effects in response to the poor larval food, although in populations not related to those studied here. In parallel to the genetic maternal effect observed here, in that study flies raised on the poor food produced offspring of a slightly smaller adult size. However, whereas the evolutionary adaptation to the poor food in the present paper was in part mediated by a genetic maternal effect on viability, Vijendravarma et al. (2010) found no plastic effect of maternal diet on offspring viability on the poor food. That study found in turn that offspring of mothers raised for one generation on the poor food developed faster on the poor food than offspring of mothers raised on standard food (Vijendravarma et al., 2010) whereas the evolution of faster development in the selected population studied here did not involve a component mediated by genetic maternal effects. Finally, we found that flies raised on poor food lay larger eggs as a plastic response (Vijendravarma et al., 2010), in contrast we have no indication that the selected population evolved larger eggs than the control population (R. K. Vijendravarma, unpublished data). Thus, although both environmental and genetic maternal effects can affect offspring fitness traits under nutritional stress, the two types of maternal effects can be quite different.

#### *Do some crosses outperform the selected parental line?*

While negative epistasis between favored alleles has been predicted by theory (see above), the magnitude of the epistatic effects is rather unexpected. In particular, larvae of some backcrosses were able to grow considerably faster than those of the Selected parental line  $P_2$ , and another apparently survived better than  $P_2$ . It is thus tempting to speculate whether some of the backcrosses actually outperform the selected parental population. A closer inspection of Figure 1 reveals that the three  $B_2$  backcrosses that reached larger adult size than  $P_2$  while developing at least as fast tended to show lower viability than  $P_2$ . In contrast, the one backcross ( $B_{2b}$ ) that tended to show higher viability than  $P_2$  had a much smaller adult weight than the other backcrosses and not different from the additive expectation. Furthermore, while fecundity in *Drosophila* typically increases with adult body size (McMillan et al., 1970), we cannot exclude trade-offs between the developmental traits we measured and adult fitness components relevant under the selection regime, or with maternal effects.

Nonetheless, it is not implausible that some admixture of genes from the unselected Control population might result in improvement of mean fitness in the selection environment compared to the parental selected population even in the absence of inbreeding depression. First, the control population may harbor some alleles that have beneficial effects on malnutrition tolerance at the genetic background of the selected population, but no or negative effects at the ancestral (control) genetic background. Alleles with this type of epistasis would likely be lost due to selection and/or drift during the initial generations of selection under malnutrition. More generally, in the presence of epistasis selection does not in general maximize mean population fitness (Gimelfarb, 1998). Second, competition often creates frequency-dependent selection, under which mean population fitness is not maximized even under additive genetics and in the absence of other genetic constraints (Abrams et al., 1993). While the selected populations are maintained at low density and the main challenge they face is the low amount of nutrients per volume of food, some competition is likely taking place under the poor food regime. Consistent with this notion, the larvae of the selected populations exert a stronger negative effect on standard competitors than the controls even under conditions where the selected and control larvae themselves survive equally well (Vijendravarma et al., 2012a). Furthermore, the selected populations have evolved a greater propensity towards larval cannibalism (Vijendravarma et al., 2013). Cannibalism in *Drosophila* seems to mostly involve pre-pupation wandering larvae being attacked by larvae less advanced in development (Vijendravarma et al., 2013). This could have generated some selection against the fastest-growing larvae, which are the first to enter the wandering stage and would thus have been at the greatest risk of cannibalism. These hypotheses remain to be tested.

### *Caveats*

While the line-cross design we used is a powerful way to detect deviations from simple additive-dominance architecture of quantitative traits, it is important to mention its limitations. First, it only describes effect averaged over all divergent loci, thus missing some potentially interesting interactions between specific loci which deviate from the overall pattern. Second, detection of epistatic interactions in our design is only possible between loci that underwent recombination in the  $F_1$  parents of second generation crosses (Lynch & Walsh, 1998). Thus, epistasis between linked loci contributes little or nothing to the observed deviations from an additive-dominance model. This could mean that the overall contribution of epistasis to the genetic architecture of divergence between the selected and the control population is underestimated. However, it is also possible that closely linked loci show synergistic epistasis more often than unlinked loci, in which case our study would overestimate the importance of antagonistic epistatic interactions between alleles favored by the malnutrition evolutionary regime. Third, the design cannot detect epigenetic effects or more complex genetic interactions, such as third- and higher-order epistatic interactions, interactions between genetic and maternal effects, or epistatic effects in mothers that affect the offspring phenotype (Lynch & Walsh, 1998; Wade, 1998; Wolf & Cheverud, 2009). Some of these issues could in principle be addressed by using more complex crossing designs, but in practice the power to detect such interaction and to distinguish between the increasing number of alternative genetic models would be weak.

Furthermore, the adaptation to malnutrition of the selected population evolved under an experimental evolution regime, involving strong selection on a rather small population. The genetic architecture of such experimentally evolved adaptations may systematically differ from evolution in nature (for an extensive discussion see Kawecki et al., 2012). In particular, it is likely to involve more genetic hitchhiking (genetic draft), whereby rather large chromosome fragments would tend to be inherited together, generating much linkage disequilibrium and accelerating loss of neutral and adaptive genetic variation (Gillespie, 2001). As a consequence, laboratory experimental adaptation in metazoans and

plants may tend to be based on fewer loci with larger allele effects than adaptation in nature (Kawecki et al., 2012).

Finally, we have only analyzed the architecture of adaptation in one of six replicate populations evolved under the malnutrition regime. Even though at the level of the phenotype these populations show parallel evolutionary changes, they may still be based on different genetic architecture, reflecting the contribution of genetic drift bringing different populations in the domains of attraction of different "adaptive peaks" (Wade & Goodnight, 1998). Such idiosyncratic responses of parallel populations have been found in some evolution experiments (e.g., Teotonio & Rose, 2000; Kawecki & Mery, 2006). Moreover, even if the responses of the replicate populations (which originated from the same base populations) were based on the same architecture, it would not mean that similar genetic architecture would apply to adaptation starting from a different initial gene pool. Thus, the assessment of the importance of epistasis and maternal effects in adaptation to nutritional stress will come from accumulation of studies in different populations, species and conditions.

### Conclusions

Unlike some other cases of experimental evolution under novel environments in insects reviewed in the introduction, this study indicates that the experimental evolutionary adaptation to chronic juvenile malnutrition in at least one population of *Drosophila* cannot be explained by a simple additive-dominance model of genetic architecture. Our results revealed strong negative (diminishing return) epistasis for alleles improving three fitness-relevant traits expressed under malnutrition. This study also showed that evolutionary change in two traits (viability and adult body weight) was to a large degree mediated by maternal effects rather than the expression of the focal individuals' own genome. Thus, with the caveats discussed above, it supports the notion epistasis and maternal effects may significantly contribute to adaptive evolution under stress.

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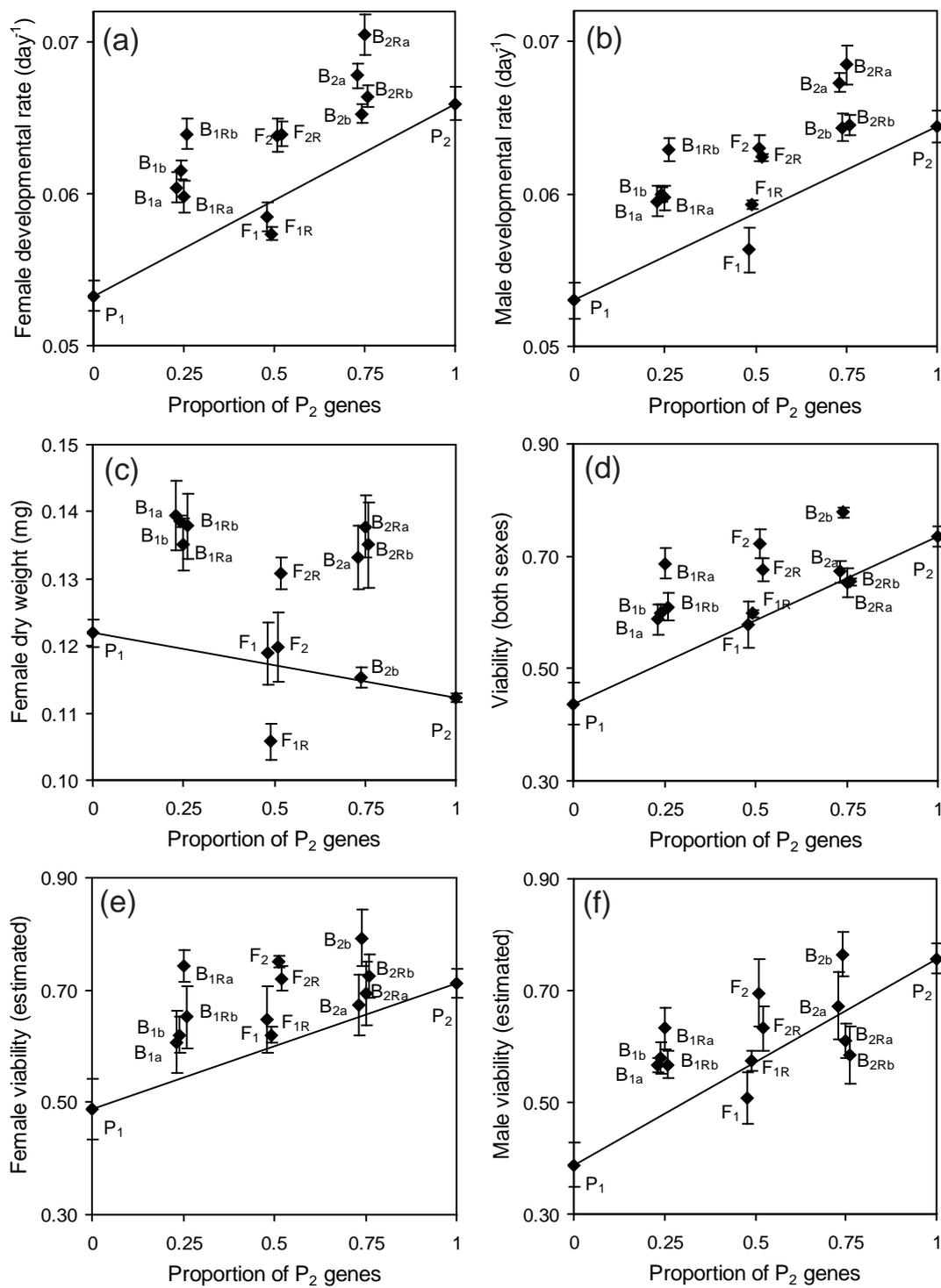
**Table 1.** Regression coefficients of the composite genetic parameters for the different types of lines. In addition, an intercept parameter  $m$  was included in all models with regression coefficient equal 1 for all lines, and where applicable, parameter  $s$  for the difference between the sexes, with coefficient  $-1$  for females and  $1$  for males.

Line	Origin	$[a]$	$[d]$	$[a \times a]$	$[a \times d]$	$[d \times d]$	$[am]$	$[dm]$	$[Xh]$	$[Xa]$	$[Xd]$	$[c]$
P <sub>1</sub>	Control line	-1	-1	1	1	1	-1	-1	-1	-1	-1	-1
P <sub>2</sub>	Selected line	1	-1	1	-1	1	1	-1	1	1	-1	1
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	0	1	0	0	1	-1	-1	-1	0	1	-1
F <sub>1R</sub>	P <sub>2</sub> × P <sub>1</sub>	0	1	0	0	1	1	-1	1	0	1	1
F <sub>2</sub>	F <sub>1</sub> × F <sub>1</sub>	0	0	0	0	0	0	1	0	-0.5	0	-1
F <sub>2R</sub>	F <sub>1R</sub> × F <sub>1R</sub>	0	0	0	0	0	0	1	0	0.5	0	1
B <sub>1a</sub>	P <sub>1</sub> × (P <sub>1</sub> × P <sub>2</sub> )	-0.5	0	0.25	0	0	-1	-1	-1	-1	-1	-1
B <sub>1b</sub>	P <sub>1</sub> × (P <sub>2</sub> × P <sub>1</sub> )	-0.5	0	0.25	0	0	-1	-1	-1	0	1	-1
B <sub>1Ra</sub>	(P <sub>1</sub> × P <sub>2</sub> ) × P <sub>1</sub>	-0.5	0	0.25	0	0	0	1	0	-0.5	0	-1
B <sub>1Rb</sub>	(P <sub>2</sub> × P <sub>1</sub> ) × P <sub>1</sub>	-0.5	0	0.25	0	0	0	1	0	-0.5	0	1
B <sub>2a</sub>	P <sub>2</sub> × (P <sub>1</sub> × P <sub>2</sub> )	0.5	0	0.25	0	0	1	-1	1	0	1	1
B <sub>2b</sub>	P <sub>2</sub> × (P <sub>2</sub> × P <sub>1</sub> )	0.5	0	0.25	0	0	1	-1	1	1	-1	1
B <sub>2Ra</sub>	(P <sub>1</sub> × P <sub>2</sub> ) × P <sub>2</sub>	0.5	0	0.25	0	0	0	1	0	0.5	0	-1
B <sub>2Rb</sub>	(P <sub>2</sub> × P <sub>1</sub> ) × P <sub>2</sub>	0.5	0	0.25	0	0	0	1	0	0.5	0	1

**Table 2.** The estimates of, and likelihood ratio tests for, the composite genetic parameters retained in the most parsimonious models. The estimates and their standard errors are expressed relative to the average of the phenotypes of the two parental lines (i.e., the means and standard errors have been divided by the "midparent value"). To facilitate the interpretation of the parameter values, the first row reports the observed difference between the two parental lines expressed on the same scale. Each parameter was tested individually ( $df = 1$ ). The significance of the three epistatic parameters ( $[a \times a]$ ,  $[a \times d]$ ,  $[d \times d]$ ) was tested jointly ( $df = 3$ ); an analogous joint test was performed on the two maternal effects parameters ( $[am]$ ,  $[dm]$ ) and, where applicable, for the three parameters describing the effect of the X chromosome ( $[Xh]$ ,  $[Xa]$ ,  $[Xd]$ ).  $R^2$  refers to the proportion of weighted sum of squares among line means explained by the model, not the proportion of variation among individuals or replicate vials.

Parameters	Developmental rate, sex-specific model ( $df = 19$ )		Female weight ( $df = 4$ )		Egg-to-adult viability, sexes pooled ( $df = 5$ )		Egg-to-adult viability, sex-specific model ( $df = 19$ )	
	Estimate $\pm$ SE	$\chi^2$	Estimate $\pm$ SE	$\chi^2$	Estimate $\pm$ SE	$\chi^2$	Estimate $\pm$ SE	$\chi^2$
$P_2 - P_1$	0.204 $\pm$ 0.018		-0.082 $\pm$ 0.019		0.507 $\pm$ 0.082		0.507 $\pm$ 0.065	
$m$	1.065 $\pm$ 0.003		1.093 $\pm$ 0.013		1.216 $\pm$ 0.029		1.173 $\pm$ 0.023	
$[a]$	0.069 $\pm$ 0.009	63.5***	–		0.075 $\pm$ 0.031	5.8*	–	
$[d]$	–		–		-0.195 $\pm$ 0.065	8.9**	-0.199 $\pm$ 0.060	11.1***
$[a \times a]$	0.032 $\pm$ 0.010	9.6**	0.127 $\pm$ 0.037	11.5***	-0.319 $\pm$ 0.128	6.2*	-0.346 $\pm$ 0.113	9.3**
$[a \times d]$	-0.014 $\pm$ 0.011	1.4	-0.029 $\pm$ 0.021	1.9	-0.087 $\pm$ 0.041	4.5*	-0.164 $\pm$ 0.036	21.1***
$[d \times d]$	-0.092 $\pm$ 0.005	283.1***	-0.154 $\pm$ 0.021	56.1***	-0.116 $\pm$ 0.041	8.0**	-0.062 $\pm$ 0.043	2.1
$[Xh]$	0.026 $\pm$ 0.005	22.9***	n/a		n/a		–	
$[Xa]$	0.012 $\pm$ 0.009	2.1	-0.023 $\pm$ 0.020	1.3	n/a		–	
$[Xd]$	0.005 $\pm$ 0.005	1.0	0.039 $\pm$ 0.014	8.3**	n/a		–	
$[am]$	–		-0.048 $\pm$ 0.013	12.9***	0.125 $\pm$ 0.021	36.1***	0.124 $\pm$ 0.023	28.0***
$[dm]$	–		0.026 $\pm$ 0.009	8.4**	-0.025 $\pm$ 0.011	5.4*	-0.006 $\pm$ 0.015	0.1
$[c]$	–		–		-0.032 $\pm$ 0.015	4.5*	-0.037 $\pm$ 0.014	7.1**
sex	-0.005 $\pm$ 0.002	3.8*	n/a		n/a		-0.048 $\pm$ 0.010	22.6***
$C_e$	0.075 $\pm$ 0.010		0.091 $\pm$ 0.018		0.276 $\pm$ 0.112		0.235 $\pm$ 0.100	
Epistasis		284.8***		58.1***		90.6***		59.3***
X-chromosome		23.0***		12.7**		n/a		5.9
Maternal effects		0.5		21.4***		110.2***		30.1***
$R^2$ / Model fit	0.91	62.8***	0.99	10.7	0.91	34.1***	0.90	30.7*

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , all other  $P > 0.09$



**Figure 1.** The mean phenotypes ( $\pm$  SE) of the two parental lines (the control line P<sub>1</sub> and the selected line P<sub>2</sub>) and twelve types of crosses between them. Under completely additive autosomal inheritance and no maternal effects the means should lie on the line connecting parental means. The bars correspond to  $\pm$  one standard error.