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Pleiotropic effects of *methoprene-tolerant* (*Met*), a gene involved in juvenile hormone metabolism, on life history traits in *Drosophila melanogaster*

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Abstract

Life history theory assumes that there are alleles with pleiotropic effects on fitness components. Although quantitative genetic data are often consistent with pleiotropy, there are few explicit examples of pleiotropic loci. The *Drosophila melanogaster* gene *Methoprene-tolerant* (*Met*) may be such a locus. The *Met* gene product, a putative juvenile hormone receptor, facilitates the action of juvenile hormone (JH) and JH analogs; JH affects many life history traits in arthropods. Here we use quantitative complementation to investigate effects of *Met* mutant and wildtype alleles on female developmental time, onset of reproduction, and fecundity. Whereas the alleles did not differ in their effects on developmental time, we detected allelic variation for the onset of reproduction and for age-specific fecundity. Alleles influenced phenotypic covariances among traits (developmental time and onset of reproduction; onset of reproduction and both early and late fecundity; early and late fecundity), suggesting that alleles of *Met* vary in their pleiotropic effects upon life history. Furthermore, the genetic covariance between developmental time and early fecundity attributed to alleles of *Met* was negative, indicating consistent pleiotropic effects among alleles on these traits. The allelic effects of *Met* support genetic models where pleiotropy at genes associated with hormone regulation can contribute to the evolution of life history traits.

Introduction

Life history traits are linked by genetical, physiological, and developmental mechanisms (e.g., Stearns, 1992) and thus cannot evolve independently. This is manifested as correlated responses to selection and genetic covariances revealed by covariances between relatives (reviewed in Roff, 1997; also see Rose & Charlesworth, 1981; Stearns & Partridge, 2001) For example, fruit flies selected for high early fecundity evolve a reduced lifespan (e.g., Partridge, Prowse & Pignatelli, 1999). However, such data are not informative about the proximate causes underlying genetic correlations because they cannot distinguish whether the correlations are caused by pleiotropy or linkage disequilibrium (e.g., Partridge & Barton, 1993).

Understanding the causes of genetic correlations and trade-offs therefore ultimately requires the identification of specific genes and their physiological effects (e.g., Silbermann & Tatar, 2000; Knight, Azevedo & Leroi, 2001; Leroi, 2001). Using molecularly characterized mutants offers a direct assessment of pleiotropic effects of single genes (e.g., van Tienderen, Hammad & Zwaal, 1996; Pigliucci, 1998; Leroi, 2001). However, although large-effect mutations and quantitative trait loci (QTL) often map to the same chromo-

somal regions (e.g., reviewed in Haag & True, 2001), such a candidate gene approach has rarely been used in evolutionary biology (e.g., Schmitt, McCormac & Smith, 1995; Pigliucci & Schmitt, 1999). Consequently, despite the long history of the pleiotropy concept in evolutionary biology (e.g., Caspari, 1952; Williams, 1957; Wright, 1968; Rose, 1985), little is known about particular pleiotropic genes affecting evolutionarily relevant traits (e.g., Barton & Turelli, 1989; van Tienderen, Hammad & Zwaal, 1996; Knight, Azevedo & Leroi, 2001). For instance, there are only few known alleles with antagonistic effects on early and late-life fitness components, which underlie the antagonistic pleiotropy theory of aging (e.g., Maynard Smith, 1958; Lin, Seroude & Benzer, 1998; Silbermann & Tatar, 2000; Arantes-Oliveira et al., 2002).

Because hormones regulate major components of life histories (e.g., growth, sexual maturity, egg production, aging), genes affecting hormone regulation have been proposed as major determinants of pleiotropy underlying life history correlations (e.g., Ketterson & Nolan, 1992; Finch & Rose, 1995; Sinervo & Svensson, 1998; Zera & Harshman, 2001). For example, juvenile hormone (JH), a sesquiterpenoid lipid-like molecule, affects many traits in insects, including metamorphosis, larval diapause regulation, vitellogenin synthesis, ovarian development, locomotor and courtship behavior, immune function, and longevity (e.g., see Nijhout, 1994; Riddiford, 1994; Wyatt & Davey, 1996; Hartfelder, 2000; Teal, Gomze-Simuta & Proveaux, 2000; Herman & Tatar, 2001; Belgacem & Martin, 2002; Rolff & Siva-Jothy, 2002).

Since manipulating and measuring JH titers is difficult, some researchers have focused on studying the effects of genetic variation in genes involved in hormone metabolism, using alleles at these loci to indirectly 'manipulate' the endocrine system. The best studied case concerns the effects of JH and JH esterase (JHE) on the trade-off between flight capability and reproduction in wing-dimorphic crickets, *Gryllus firmus* (e.g., Zera & Huang, 1999; Zhao & Zera, 2002). There are also several lines of evidence suggesting that JH may exert multiple phenotypic effects on *Drosophila* life history (e.g., see Templeton & Rankin, 1978; DeSalle & Templeton, 1986; Tatar & Yin, 2001; Tatar, Chien & Priest, 2001; Tatar, Bartke & Antebi, 2003). For example, some *Drosophila melanogaster* mutant genotypes of the *Insulin-like Receptor* (InR) gene produce infertile dwarf females with nonvitellogenic ovaries and delayed senescence, extending lifespan by up to 85% (Tatar et al., 2001). The activity of the corpora allata, the glands synthesizing JH, is reduced in these flies. Application of methoprene, a JH analog, restores egg development and fecundity and reduces life expectancy to that of the wildtype. This suggests that JH is involved in mediating the trade-off between reproduction and longevity (e.g., Tatar & Yin, 2001; Tatar et al., 2001; Tatar, Chien & Priest, 2001; Tatar, Bartke & Antebi, 2003).

Thus, hormones, for example JH, are thought to mediate genetic correlations and trade-offs between life history traits, coordinating their integrated expression across environmental conditions or constraining their evolution. Consequently, we may expect that loci associated with hormone metabolism will exhibit pleiotropy because alleles at such loci affect the regulation of hormones, which, in turn, have multiple phenotypic effects (Rose, 1991; Finch & Rose, 1995; Sinervo & Svensson, 1998). Furthermore, alleles may vary in their pleiotropic effects upon life history because they differ in how they regulate the hormonal machinery that coordinates trait expression. Thus, as suggested by Zera and Harshman (2001), future studies of evolutionary endocrinology should extend to include, for example, the regulation of life history traits at the level of hormone receptors. Our present study is an attempt to do so. If the pleiotropy at endocrine loci is ultimately due to alleles affecting the regulation of hormones with multiple phenotypic effects (hormonal pleiotropy, Finch & Rose, 1995), we may expect that loci coding for JH-binding proteins or receptors affect multiple life history traits. The X-linked *D. melanogaster* gene *Methoprene-tolerant* (*Met*) is such a candidate locus.

Met codes for a transcription factor which is a high-affinity JH binding protein. Although there are only minor differences in the penetration, excretion, tissue sequestration, and metabolism of JH between *Met* mutants and wildtype strains (Shemshedini & Wilson, 1990), some mutants have reduced JH binding affinities. Consequently, mutants can survive exposure to pharmacological doses of JH (or the JH analog methoprene) that are lethal to wildtypes (e.g., Wilson & Fabian, 1986; Shemshedini & Wilson, 1990). Many prop-

erties of the MET protein (saturability, high JH binding affinity, JH ligand specificity, tissue specificity, stimulation of protein synthesis) suggest that it is a JH receptor (e.g., Shemshedini, Lanoue & Wilson, 1990). Yet, in contrast to what one would expect from a JH receptor, null mutations of *Met* are viable (Wilson & Ashok, 1998; see also Truman & Riddford, 2002). Furthermore, some of the tissues in which MET is present are known JH targets, yet others are not (Pursley et al., 2000). Thus it is currently not clear whether MET is a JH receptor or whether it represents another type of JH binding protein. Although at present the exact molecular nature of the JH receptor remains unclear (e.g., Gilbert, Granger & Roe, 2000; Truman & Riddiford, 2002), the *Met* locus appears to be intimately involved in JH metabolism (e.g., Ashok, Turner & Wilson, 1998; Restifo & Wilson, 1998; Pursley, Ashok & Wilson, 2000).

Some mutant alleles at this locus strongly reduce the number of vitellogenic oocytes and eggs a female can produce (e.g., Minkoff & Wilson, 1992; Wilson & Ashok, 1998), consistent with the physiological model of JH effects on vitellogenesis and oogenesis (e.g., Bownes, 1982; Wilson, Landers & Happ, 1983; Soller, Bownes & Kubli, 1999). Although preliminary evidence suggests that *Met* may also affect other life history traits (Minkoff & Wilson, 1992), reported effects were weak and likely to be confounded by effects of genetic background.

Here we test whether mutant and wildtype alleles at the *Met* locus have pleiotropic affects on life history traits. Since *Met* is a X-linked gene we are specifically interested in life history traits of females. In particular, we ask whether (i) allelic variation at *Met* affects two or more traits (pleiotropy), (ii) whether *Met* alleles affect phenotypic covariances (variation for pleiotropy), (iii) whether pleiotropic allelic effects are correlated (intralocus genetic covariance), and (iv) whether these correlations suggest the presence of trade-offs mediated by the *Met* locus.

We use quantitative complementation testing (QCT; Mackay & Fry, 1996) to quantify the effects of *Met* mutant and wildtype alleles on female developmental time, onset of reproduction, early and late fecundity, and on the relationships among these traits. This method allows us to (i) detect variation and covariation among life history traits induced by *Met* alleles with small, quantitative heterozygous effects, and (ii) to control for confounding effects of loci in the genetic background of the *Met* locus.

Methods

Quantitative complementation tests

QCT tests whether a particular candidate locus (the test locus) contributes to phenotypic variation of a given trait (e.g., Long et al., 1996; Mackay & Fry, 1996; Gurganus et al., 1999; Palsson & Gibson, 2000). Specifically, QCT estimates the relative genetic variance when the 'variable' alleles (a_i) are complemented to standard alleles in defined genetic backgrounds. The variable alleles are sampled from populations or from mutant collections. Standard alleles occur in two 'complementation haplotypes', i.e., the chromosal stocks into which each of the variable alleles is crossed: a 'mutant complement' and a 'wildtype complement'. Each variable allele is crossed into a stock carrying a null or hypomorphic allele at the test locus (the mutant complement, $-$) and into a stock carrying a reference wildtype allele at the test locus (the wildtype complement, $+$). Thus, there are two crosses for each variable allele: a 'tester' cross ($t_i = a_i/-$) in which the variable allele is in heterozygous state with the mutant complement and a 'control' cross ($c_i = a_i/+$) in which the variable allele is in heterozygous state with the wildtype complement. Phenotypes are measured for a series of variable alleles in both complementation states. Allelic variation at the test locus is estimated by a significant interaction of the variable allele term and the complementation haplotype term in an analysis of variance; this is equivalent to testing for significant variation in $t_i - c_i$ values among variable alleles (e.g., Long et al., 1996; Mackay & Fry, 1996). In particular, pleiotropy at the test locus is detected by simultaneously testing for significant interactions of the variable allele term and the complementation haplotype term for multiple traits, i.e. there is variation in $t_i - c_i$ values among variable alleles for several traits. Covariance among traits attributed to segregation of alleles at the test locus is estimated by a significant correlation of $t_i - c_i$ values among traits. In this study, we shall determine the variance and covariance for developmental and reproductive traits among alleles of *Met*.

To estimate variation in heterozygous effects at the test locus we require that the background effects do not affect our estimate of allelic variation. Here, background variation is controlled in three ways. First, at each variable allele, additive effects of background loci of the chromosome that carries the allele are substracted out because the same chromosomes are used in both the tester and the control cross. (e.g., Long et al., 1996; Mackay & Fry, 1996). Furthermore, five of the variable alleles we tested and the mutant complement originated from the same progenitor strain (*vermilion*; see below) and have therefore similar genetic backgrounds. Second, QCT assumes that there is no epistasis between loci in the background and the focal test locus (e.g., Long et al., 1996; Mackay & Fry, 1996). In our experiment we know *a priori* that the variable alleles used are alleles of *Met*. Thus, the most parsimonious explanation for detecting significant allele by complementation haplotype interactions is that the effects of the variable *Met* alleles depend on the complementation haplotype present at *Met*, not on epistasis between the test locus and loci in the genetic background of *Met*. Third, the genetic background does not affect var($t-c$) if the complementation haplotypes are, except at the test locus, genetically identical (e.g., Long et al., 1996; Mackay & Fry, 1996). Therefore, we introduced the complementation haplotypes into a common background.

Fly strains and culture conditions

We assayed the allelic effects of six *Met* mutant alleles (Met^{1}, *yv* Met^{3}, *v* Met^{N6}, *v* Met^{D29}, *v* Met^{128}, Met^{E1}), one transgenic strain with a partial rescue of function (*wv* Met^{27}; *p*[w^{+} *EN71*]/+), and three wildtype strains (*B*, *F*, *3*). We included both mutant and wildtype alleles to 'catch' a wide range of allelic effects and to check qualitatively for putative deleterious effects of mutant alleles as compared to the presumably less deleterious effects of wildtype alleles. The transgenic strain was included because it may provide an 'internal' control that can be used to validate the QCT method in terms of specifically detecting allelic effects at the *Met* locus (see 'Statistical Analysis').

All mutant strains and the transgenic line originated from a *yellow vermilion* (*yv*), *white vermilion* (*wv*) or *vermilion* (*v*) background, except for Met^{1} and Met^{E1} which originate from an Oregon-RC background. The *vermilion* gene is a convenient marker, closely linked to *Met*. The *Met* mutant alleles are either EMS (ethyl-methanesulfonate)-induced or X-ray-induced alleles, showing a methoprene-resistance phenotype (e.g., Wilson & Fabian, 1986; Ashok, Turner & Wilson, 1998; details on *Met* alleles at http://flybase.bio.indiana.edu, accession FBgn0002723). The rescue strain had a *P*-element insert including a functional copy of Met^{+}, *p*[w^{+} *EN71*] (Ashok, Turner & Wilson, 1998), which restores sensitivity to methoprene and eliminates the ovipositional defect observed in *wv* Met^{27} null mutants (Wilson & Ashok, 1998). The three wildtype strains *B*, *F*, and *3* had *Met* wildtype alleles and were highly sensitive to methoprene (Flatt, unpublished data). The *B* strain was produced in September 2000 by intercrossing 40 independent isofemale lines (collected by S.C. Stearns et al. near Basel, Switzerland, Summer 1986); the *F* strain was initiated with 2000 wild-caught flies (collected by F. Mery, Basel, June 2000), and the *3* strain is an isogenic laboratory strain (initiated by N. Vouilloz, Basel, September 2000). As the mutant complement we used the null allele, *wv* Met^{27} (Ashok, Turner & Wilson, 1998; Wilson & Ashok, 1998), which is maintained over a *FM7a* balancer. As the wildtype complement we used an isogenic laboratory wildtype strain, *STD* (initiated by N. Vouilloz, Basel, September 2000).

All stocks were maintained at 25°C, with a relative air humidity of 70%, and a 12L:12D photoperiod. Stocks were cultured in 175-ml polystyrol culture bottles on a standard yeast–cornmeal–agar medium and transferred to new bottles every 2–3 weeks.

Complementation haplotypes

To homogenize the genetic background among the complementation haplotypes we extracted the chromosomes carrying the mutant and the wildtype complement into a common autosomal background. To achieve this, 250 wildtype males of the *STD* wildtype complement strain (X_{STD} / Y_{STD}) were mass-mated with 250 females of the mutant complement strain (*wv* Met^{27}/*FM7a*). Thus, the F1 of this cross had 50% of its autosomes from the *STD* strain and 50% from the *wv*

Met^{27} strain, and subsequent generations created recombinant parental chromosomes which further homogenized variation due to the genetic background. From this cross, we established two new cultures, a new *STD* wildtype complement culture and a new *wv* Met^{27} mutant complement culture, both having a common autosomal background.

To initate the new *STD* culture, we selected $X_{STD}/FM7a$ females and $FM7a/Y_{STD}$ males in the F1 of the above-mentioned cross. Subsequently, from this new culture, we selected in each generation X_{STD} /*FM7a* females and $FM7a/Y_{STD}$ males to breed through, maintaining the wildtype complement X_{STD} chromosome over the same *FM7a* balancer that was used for the mutant complement. This procedure was repeated for 10 generations until the start of the experiment. Similarly, to initiate the new *wv* Met^{27} culture, we selected *wv* $FM7a/X_{STD}$ females and *wv* Met^{27}/Y_{STD} males from the F2 in the above-mentioned cross. These flies were allowed to breed through in the new culture. From the mating of these genotypes we selected, for 10 generations, in each generation $FM7a/wv\ Met^{27}$ females and $FM7a/\ Y_{STD}$ males.

Design of QCT crosses

For any given variable allele (Met_i), we used the following crossing schemes to obtain the tester and the control cross:

Tester: Met^{27}/Met^{27} females
$\times\ Met_i/$Ymales, resulting in: Met_i/Met^{27},
Control: X_{STD}/X_{STD} females
$\times\ Met_i/$Y males, resulting in: Met_i/X_{STD}.

To set up the 20 crosses (mutant versus wildtype complement ×10 variable alleles) we used virgin females and unmated males collected over an eight-day period, and stored these flies at 18°C to minimize physiological differences among flies collected earlier or later. The crosses were set up at 25°C in culture bottles. For each of the 10 variable allele strains, one bottle with 20 *wv* Met^{27}/wv Met^{27} and one bottle with 20 X_{STD}/X_{STD} virgin females was set up, and to each of these bottles 20 unmated males of the variable allele strain were added. After three days, the flies were transferred to new culture bottles with attached petri dishes containing 10 ml of food medium sprinkled with live yeast, and allowed to oviposit overnight. Two samples of 50 eggs were collected from each cross and transferred to two vials, each containing 5 ml of food medium. The 40 vials (10 variable alleles ×two crosses ×two replicate vials) were kept in a climate chamber (25°C, 70% relative humidity, 12L:12D); the position of the rack containing the vials was changed daily.

Assays of fitness components

Virgin females were collected within 12 h of eclosion until all flies had emerged. Each virgin was transferred individually with two adult *STD* males to a 10 ml plastic vial with 2 ml food medium. The lids with medium were replaced every second day over the next 24 days; eggs laid during each 48-h period were counted. Onset of reproduction (= age at maturity) was defined as the time between eclosion and first oviposition (resolution: 2 days). Age-specific daily fecundity was estimated as half the average number of eggs laid per female during a 48-h interval. When estimating average fecundity, fecundity was averaged over all reproductive females alive in a given 48-h interval. We note that our fecundity estimates were rather low (cf. also Tatar et al., 1996). This was expected since the number of eggs laid per female depends positively on the density of ovipositing females and the presence of live yeast (e.g., Ashburner, 1989). Although in our experiment females were kept for practical reasons singly on food medium that was not supplemented with live yeast, our estimates agree well with those obtained by Rockwell and Grossfield (1978) for females kept singly on non-nutrient agar. Thus, our egg count data represent conservative, minimal estimates of fecundity. When exchanging lids we also checked for dead flies, replacing dead males if necessary. Sample sizes of the life history measurements in the tester and control crosses for the different alleles are given in the Appendix.

Statistical analysis

To investigate allelic effects on life history traits we used several uni- and multivariate linear models. The data were analyzed using JMP IN 3.2.6. (SAS Institute; Sall & Lehman, 1996) and SAS versus 8.0 (SAS Institute 2000).

We checked for approximate normality by visual inspection of residuals and for homoscedas-

ticity using both O'Brien's and Brown-Forsythe's test for homogeneity of variances (e.g., Sall & Lehman, 1996). Because data were in some cases heteroscedastic or residuals were not normally distributed, we checked the robustness of our results by analyzing Box-Cox transformed data. The Box–Cox method finds the best transformation in terms of normality and homoscedasticity (Sokal & Rohlf, 1995). The results from analyses based on untransformed versus Box-Cox transformed data usually agreed well, indicating that results are robust and that deviations from normality and homoscedasticity were unlikely to be a problem when analyzing untransformed data. Nonetheless, because results sometimes differed between the two types of analysis, we report results from both analyses.

QCT data were analyzed using linear models that share the following structure (e.g., Long et al., 1999):

$$X_{ijkl} = \mu + A_i + B_j + AB_{ij} + C(AB)_{k(ij)} + e_{l(ijk)}, \quad (1)$$

where X_{ijkl} represents the value of the dependent variable for any replicate vial k for a given variable allele i and cross j, μ is the overall mean of the dependent variable, A_i the effect of the ith variable allele (fixed factor), B_j the cross effect, i.e. the effect of crossing the variable alleles to the mutant complement versus the wildtype complement (t_i = tester, c_i = control; fixed factor), AB_{ij} the variable allele by cross interaction (fixed), $C(AB)_{k(ij)}$ the effect of replicate vials nested in combinations of cross and variable allele (random), and $e_{l(ijk)}$ the residual (error) term. The main effect of variable allele averaged over the tester and control cross tests the mean effect of a variable allele and of the genetic background of the chromosome carrying the variable allele. The main effect test of cross (tester versus control) averaged over all variable alleles tests the mean effect of the mutant complement to wildtype complement substitution. Most importantly, a significant AB_{ij} interaction term indicates that the effects of variable alleles depend on the complementation haplotype involved in the crosses. Thus, a significant allele by cross interaction suggests that alleles differ in their heterozygous effects on a particular trait, i.e., that $\mathrm{var}(t_i - c_i) > 0$ (see above). The factors A_i and B_j and the interaction AB_{ij} were tested over the $C(AB)_{k(ij)}$ term, whereas the $C(AB)_{k(ij)}$ term was tested over the residual. As outlined below, the above model structure was used in several statistical tests.

Multivariate and univariate allelic effects

We first considered all 10 variable alleles and investigated their multivariate and univariate allelic effects using the above model structure in three different analyses, (1)–(3) (see below). Since we were particularly interested in detecting allelic variation, we first tested in all three analyses for significant main effects of the variable allele. Yet, the main effects of variable allele may be confounded by effects of genetic background, whereas the AB_{ij} interactions are unlikely to be confounded by such effects. We thus tested in all three analyses for significant variation among all *Met* alleles in their effects on life history traits as revealed by significant AB_{ij} interactions. Finally, we then tested in analyses (1)–(3) for pleiotropy at the *Met* locus by simultaneously testing for significant AB_{ij} interactions for multiple traits.

(1) Because many traits measured were correlated, we used multivariate analysis of variance (MANOVA) to test for allelic variation and pleiotropy for the multivariate phenotype (linear combination of developmental time, onset of reproduction, early and late fecundity) before proceeding to ANOVAs for individual traits. (2) For each trait, we performed analysis of variance (ANOVA), again testing for allelic variation and pleiotropy. We analyzed three measures of fecundity: early fecundity (daily per capita fecundity averaged over the first 10 days after onset of reproduction), late fecundity (average fecundity between days 10 and 20), and total fecundity (fecundity between onset of reproduction and when a female's last eggs were collected). (3) Because fecundity from one day to the next may be correlated within subjects, we also used repeated measures MANOVA (e.g., von Ende, 2001) to test for main and interaction effects while controlling for within-subject covariance. Consequently, among-subject effects (A_i, B_j, $C(AB)_{k(ij)}$, AB_{ij}) and within-subject effects (time) were analyzed coordinately using F values based on Roy's greatest root (e.g., Harris, 1985). Here a significant time by AB_{ij} interaction indicates that variable

alleles differ in their effects on fecundity trajectories over time.

Effects of mutant versus wildtype alleles

By lumping together all wildtype and mutant alleles for analysis one assumes that all alleles come from the same population of potential alleles for a given locus and that their effects share a common distribution. However, this may not be the case because mutant alleles, isolated from mutagenesis screens, are likely to show relatively strong developmental genetic effects, whereas wildtype alleles may presumably exhibit milder effects.

(1) To test whether variation in allelic effects differs between the mutant and wildtype allele subsamples, we calculated contrasts for the AB_{ij} interaction from factorial QCT ANOVAs (cf. Equation (1)). (2) Because results confirmed that mutants and wildtypes differed in their allelic effects, we proceeded to univariate ANOVAs, analysing the QCT data separately for mutant and wildtype alleles and testing for significant AB_{ij} interactions within these two categories. (3) Assuming that (i) the wildtype complement is dominant to all alleles, (ii) the mutant complement is recessive to all alleles and (iii) mutant alleles differ on average in their allelic effects from wildtype alleles, we predicted that allelic effects (and differences between mutant and wildtype effects) may be stronger in tester crosses (because of the recessivity of the mutant complement) than in the control crosses (because of the dominance of the wildtype complement). This hypothesis was tested by calculating, separately for tester and control crosses, mutant-wildtype contrasts for the main effect of allele in two-way ANOVAs with the factors allele (A_i) and vial nested in allele $C(A)_{k(i)}$.

Effects of the transgene versus wildtype alleles

We included one transgenic rescue strain, *wv* Met^{27}; $p[w^+\ EN71]/+$, to control for how specific the QCT method is with regard to detecting allelic effects at a single locus. Thus, if the transgene provides a rescue of MET function, the heterozygous allelic effects of the transgene, $h_{i=n}$, may be expected to be (i) the same for the tester and the control cross (because of the dominance of the transgenic wildtype allele and the wildtype complement) and (ii) on average not different from the effects of the wildtype alleles.

(1) We tested for a significant effect of cross in univariate ANOVAs on QCT data for the transgene using cross (B_j) and vial nested in cross $[C(B)_{k(j)}]$ as factors. The absence of a difference between tester and control would confirm our expectation that the transgene provides a functional rescue. (2) We predicted that variation in allelic effects does not differ between the transgene and the wildtype alleles. We tested this hypothesis by calculating interaction contrasts from the fully factorial univariate QCT ANOVA models, excluding all the data on mutant alleles. (3) We predicted that the effects of the transgene and wildtype alleles are on average the same in both control and tester crosses, assuming that (i) the wildtype complement is dominant, (ii) the mutant complement is recessive, (iii) the transgene provides a rescue of MET function, and (iv) the transgenic wildtype allele shows a high degree of dominance. If these assumptions are met, one expects that allelic effects are on average the same in tester crosses (because of the dominance of both the wildtype alleles and the transgenic wildtype allele as compared to the recessive mutant complement) and control crosses (because of the dominance of the wildtype alleles, the transgenic wildtype allele, and the wildtype complement). We tested this prediction by calculating, separately for tester and control, transgene versus wildtype contrasts for the main effects of allele in univariate QCT ANOVAs using allele (A_i) and vial nested in allele $[C(A)_{k(i)}]$ as factors.

Allelic effects on trait relationships

Finally, we tested for (i) allelic contributions to phenotypic covariances among traits and for (ii) genetic correlations among traits attributed to segregation of alleles at the test locus.

(1) Because traits were phenotypically correlated, we tested for allelic effects on a given trait while controlling for variation in another trait (covariate) using analysis of covariance (ANCOVA). The assumption of homogeneity of slopes was tested as an interaction between model effects and the covariate. In particular, a significant interaction between the covariate and the AB_{ij} term indicates that the slopes of the regression lines for the two traits (i.e., covariate and depen-

dent variable) differ between the tester and the control crosses and that this $t-c$ difference in slopes differs among alleles. Thus, rather than testing for allelic variation in single independent traits, we asked whether alleles differ in their effects on the linear phenotypic relationship between traits. Specifically, a significant covariate $\times AB_{ij}$ interaction suggests that the effects on phenotypic covariances differ among alleles, indicating allelic variation for pleiotropic effects. Such variation would suggest that alleles do not have consistent pleiotropic effects, i.e., alleles differ on average in the signs and magnitudes of their pleiotropic effects (see below). (2) We estimated intralocus genetic correlations, defined as genetic correlations between two traits for allelic effects at a particular locus (e.g., Leroi, 2001), using parametric correlations. A significant correlation suggests that alleles have on average consistent pleiotropic effects, indicating that alleles are on average either significantly positively ($++$, $--$) or negatively (antagonistically; $+-$, $-+$) pleiotropic. In particular, a significant negative genetic correlation between fitness components may be taken as an indication that the alleles at the test locus contribute to a trade-off.

Results

Multivariate and univariate allelic effects

The untransformed life history data for the variable alleles are summarized in Figure 1.

(1) The MANOVA revealed significant effects of variable allele (Roy's greatest root = 7.33, approx. $F_{9,20} = 16.28$, $p < 0.0001$) – indicative of allelic variation, cross (Roy's greatest root = 2.13, $F_{4,17} = 9.04$, $p = 0.0004$) and vial (Roy's greatest root = 0.07, approx. $F_{20,707} = 2.59$, $p = 0.0002$). Most importantly, *Met* alleles also differed in their effects on the multivariate phenotype (AB_{ij} interaction, Roy's greatest root = 8.34, approx. $F_{9,20} = 18.53$, $p < 0.0001$). A MANOVA on Box-Cox transformed data yielded qualitatively similar results (not shown; in all cases $p < 0.01$).

(2) Table 1 summarizes the results of the factorial ANOVAs. We detected significant allelic variation in phenotypic effects for multiple traits (main effect of allele, cf. Table 1). However, the main effects of variable allele may be confounded by effects of genetic background. We also found several significant allele × cross interactions, which are unlikely to be confounded by background effects, thus confirming that the *Met* locus affects multiple traits. First, alleles did not differ in their effects on developmental time (Figure 1(A), Table 1). Second, while the analysis of untransformed data suggested that variation among alleles did not affect the onset of reproduction (Table 1, Figure 1 (B)), analysis of transformed data showed that alleles differed in their effect on this trait (Table 1, Box-Cox transformed data, AB_{ij} interaction: $F_{9,20.50} = 2.95$, $p = 0.020$). Third, variation among alleles had marked effects on different measures of fecundity. We detected significant allelic variation for early fecundity (Table 1, Figure 1 (C)). Similarly, although the AB_{ij} interaction was marginally non-significant for late fecundity (Table 1, Figure 1 (D)), analysis of transformed data revealed significant allelic variation for this trait (Table 1, Box-Cox transformed data, AB_{ij} interaction: $F_{9,21.09} = 2.93$, $p = 0.020$). Furthermore, alleles differed in their effects on total fecundity (Table 1, Figure 1 (E)).

(3) The repeated measures MANOVA on fecundity data (Table 2) revealed a significant effect of the AB_{ij} interaction when controlling for within-subject covariance, confirming that variable alleles differed in their effects on fecundity. We also found a significant time $\times AB_{ij}$ interaction, indicating that alleles differ in their effects on fecundity trajectories over time.

Effects of mutant versus wildtype alleles

(1) The 'mutant versus wildtype' interaction contrast was significant for early fecundity, suggesting that the two groups of alleles differed in their effects on this trait (Table 1, Figure 1). In contrast, the two allelic categories did not differ in their effects on developmental time, onset of reproduction, late and total fecundity (Table 1, Figure 1), suggesting that the effects of mutant versus wildtype allelic variation are qualitatively similar for most traits. Accordingly, when testing for the interaction effect while accounting for the 'mutant versus wildtype' contrast, alleles differed significantly from each other in their effects on early, late, and total fecundity (significant residual interaction contrasts, Table 1, Figure 1), indicating that allelic variation can typically not be explained

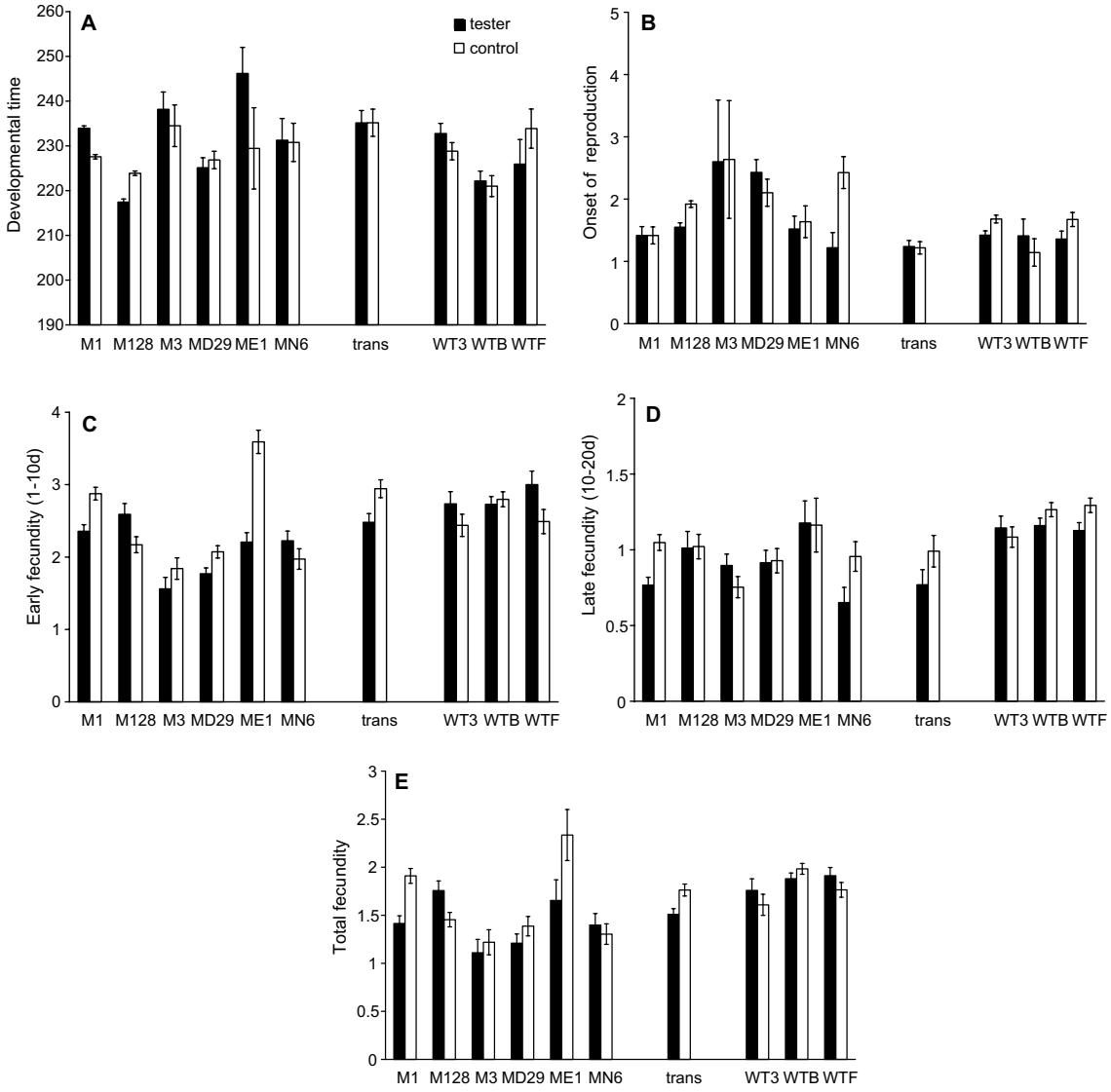

Figure 1. Untransformed life history data. For all alleles, the mean trait value (±1 SE) in the tester and the control cross is plotted for each trait. For a given allele, the difference between the tester and the control cross (t−c) is an estimate of the heterozygous allelic effect. Negative t−c values ($t < c$) indicate that a variable allele decreases a trait and positive values ($t > c$) indicate that a variable allele increases a trait relative to the control cross. (A). Developmental time. (B). Onset of reproduction. (C). Early fecundity between day 1 and 10. (D). Late fecundity between day 10 and 20. (E). Total fecundity. See text for definitions of traits and the Appendix for sample sizes. *trans.* = transgene, *WT* = wildtype.

by differences between mutant versus wildtype alleles.

(2) Among mutant alleles, we found significant allelic variation for early (Table 3, Figure 1(C)), late (Figure 1(D)), and total fecundity (Figure 1(E)). Among wildtype alleles, in contrast, alleles only differed in their effects on the onset of reproduction (Table 3, Figure 1(B)), whereas other fitness components were not affected. Interestingly, for mutant alleles, the average developmental time was significantly longer for the tester than for the control crosses (Table 3, Figure 1(A)). In contrast, for early and total fecundity, individuals in tester crosses had on average a significantly lower fecundity than those in the control crosses (Table 3, Figure 1(C), and (E)). Thus, the longer

Table 1. Results of univariate ANOVAs on life history trait data for all alleles, including the transgene

Source	Develop. time	Onset of reprod.	Fec. (1—10 days)	Fec. (10—20 days)	Fec. total
Allele (A_i)	$F_{9,\ 20.45} = 4.56$ $^*p = 0.0021$	$F_{9,\ 20.29} = 3.69$ $^*p = 0.007$	$F_{9,\ 20.82} = 17.10$ $^*p < 0.0001$	$F_{9,\ 21.02} = 6.20$ $^*p = 0.0003$	$F_{9,\ 20.38} = 10.18$ $^*p < 0.0001$
Cross (B_j)	$F_{1,\ 20.52} = 1.19$ $p = 0.29$	$F_{1,\ 20.51} = 1.41$ $^*p = 0.25$	$F_{1,\ 21.59} = 6.74$ $^*p = 0.017$	$F_{1,\ 22.71} = 4.65$ $^*p = 0.042$	$F_{1,\ 20.69} = 5.09$ $^*p = 0.035$
AB_{ij}	$F_{9,\ 20.45} = 1.33$ $p = 0.28$	$F_{9,\ 20.29} = 1.24$ $^*p = 0.33$	$F_{9,\ 20.82} = 9.39$ $^*p < 0.0001$	$F_{9,\ 21.02} = 2.19$ $^*p < 0.066$	$F_{9,\ 20.38} = 3.54$ $^*p = 0.0086$
A_i (M versus WT)B_j	$F_{1,\ 18.40} = 0.67$ $p = 0.42$	$F_{1,\ 18.30} = 0.10$ $p = 0.75$	$F_{1,\ 18.85} = 14.95$ $^*p = 0.0011$	$F_{1,\ 19.13} = 0.01$ $p = 0.92$	$F_{1,\ 18.42} = 4.10$ $p = 0.058$
A_i (residual)B_j	$F_{7,\ 18.40} = 1.47$ $p = 0.16$	$F_{7,\ 18.30} = 1.39$ $p = 0.27$	$F_{7,\ 18.85} = 9.39$ $^*p = 0.0006$	$F_{7,\ 19.13} = 2.93$ $^*p = 0.029$	$F_{7,\ 18.42} = 4.05$ $^*p = 0.0078$
Vial (AB_{ij})	$F_{20,\ 1034} = 4.18$ $^*p < 0.0001$	$F_{20,\ 810} = 2.33$ $p = 0.0009$	$F_{20,\ 808} = 0.85$ $p = 0.65$	$F_{20,\ 708} = 0.76$ $p = 0.76$	$F_{20,\ 810} = 1.82$ $^*p = 0.015$

To check the robustness of results we repeated the analysis using Box-Cox transformed data (details not shown; *refers to $p < 0.05$ on Box–Cox transformed data). The interaction contrasts A_i (M versus WT)B_j test whether interaction effects differ between mutant and wildtype alleles; the A_i (residual)B_j contrasts test for significant interaction effects after the A_i (M versus WT)B_j contrast has been accounted for. Data for the transgenic line were excluded from the contrast analysis.

Table 2. Results of repeated measures MANOVA for fecundity data

Source	Roy's greatest root	F	d.f. $_{num.}$	d.f. $_{den.}$	P
Between subjects					
Allele (A_i)	7.35	16.33*	9	20	<0.0001**
Cross (B_j)	0.58	1.44	6	15	$=0.2643$
AB_{ij}	6.01	13.36*	9	20	<0.0001**
Vial (AB_{ij})	0.06	2.00*	20	636	<0.0060**
Within subjects					
Time	3.97	502.32	5	632	<0.0001**
Time$\times A_i$	0.12	8.46*	9	636	<0.0001**
Time$\times B_j$	0.006	0.70	5	632	$=0.6217$
Time$\times$Vial (AB_{ij})	0.05	1.62*	20	636	$=0.0429$**
Time$\times AB_{ij}$	0.12	8.75*	9	636	<0.0001**

The fecundity data were partioned into 6 fecundity estimates over 4 days (i.e., daily per capita fecundity between days 1–4, 4–8, 8–12, etc.).
*refers to approximate F values.
**refers to $p < 0.05$ in the analysis of Box–Cox transformed data.

developmental time and the lower early and total fecundity in the tester as compared to the control crosses suggests that mutant alleles exhibit partially recessive deleterious fitness effects in tester crosses, whereas these effects are partially masked by the wildtype complement in the control crosses. Consistent with this hypothesis, we found that the main effects of cross were much less pronounced among wildtype than among mutant alleles (Table 3, Figure 1). The absence of differences between tester and control crosses for wildtype alleles may therefore indicate that the wildtype alleles are on average functionally equivalent, showing a high degree of dominance and presumably having similar effects on fitness.

(3) As expected, for tester crosses, mutants and wildtypes differed in their effects on several traits, i.e. for early, late, and total fecundity (details of ANOVA not shown, cf. Figure 1; contrasts, in all cases: $F_{1,8} > 20.0$, $p < 0.01$). For control crosses,

Table 3. Results of univariate ANOVAs, analyzing mutant and wildtype alleles separately and excluding the data for the transgenic line

Source	Develop. time	Onset of reprod.	Fec. (1–10d)	Fec. (10–20d)	Fec. total
Mutant alleles					
Allele (A_i)	$F_{5,\ 12.26} = 4.70$	$F_{5,\ 12.24} = 2.38$	$F_{5,\ 12.97} = 25.01$	$F_{5,\ 12.90} = 4.55$	$F_{5,\ 12.35} = 11.06$
	$^*p = 0.013$	$^*p = 0.1$	$^*p = 0.0001$	$^*p = 0.013$	$^*p = 0.0003$
Cross (B_j)	$F_{1,\ 12.29} = 1.70$	$F_{1,\ 12.40} = 0.92$	$F_{1,\ 13.74} = 16.16$	$F_{1,\ 13.84} = 1.80$	$F_{1,\ 12.59} = 6.16$
	$p = 0.012$	$^*p = 0.36$	$^*p = 0.0013$	$p = 0.20$	$^*p = 0.028$
AB_{ij}	$F_{5,\ 12.26} = 1.50$	$F_{5,\ 12.24} = 1.20$	$F_{5,\ 12.97} = 14.36$	$F_{5,\ 12.87} = 3.09$	$F_{5,\ 12.35} = 4.46$
	$p = 0.26$	$^*p = 0.37$	$^*p < 0.0001$	$^*p = 0.047$	$^*p = 0.015$
Vial (AB_{ij})	$F_{12,\ 633} = 5.80$	$F_{12,\ 477} = 2.56$	$F_{12,\ 475} = 0.68$	$F_{12,\ 399} = 0.84$	$F_{12,\ 477} = 1.84$
	$^*p < 0.0001$	$p = 0.0027$	$p = 0.76$	$p = 0.60$	$p = 0.039$
Wildtype alleles					
Allele (A_i)	$F_{2,\ 6.14} = 3.77$	$F_{2,\ 6.22} = 5.15$	$F_{2,\ 6.09} = 0.48$	$F_{2,\ 6.30} = 1.78$	$F_{2,\ 6.10} = 3.13$
	$p = 0.089$	$^*p = 0.048$	$p = 0.64$	$p = 0.24$	$p = 0.12$
Cross (B_j)	$F_{1,\ 6.15} = 0.005$	$F_{1,\ 6.24} = 2.24$	$F_{1,\ 6.10} = 3.23$	$F_{1,\ 6.31} = 2.49$	$F_{1,\ 6.10} = 0.50$
	$p = 0.95$	$p = 0.17$	$p = 0.12$	$p = 0.16$	$p = 0.51$
AB_{ij}	$F_{2,\ 6.14} = 1.50$	$F_{2,\ 6.22} = 6.50$	$F_{2,\ 6.09} = 1.62$	$F_{2,\ 6.30} = 1.61$	$F_{2,\ 6.10} = 1.01$
	$p = 0.30$	$^*p = 0.0299$	$p = 0.27$	$p = 0.27$	$P = 0.42$
Vial (AB_{ij})	$F_{6,\ 300} = 2.85$	$F_{6,\ 245} = 0.48$	$F_{6,\ 245} = 1.17$	$F_{6,\ 228} = 0.44$	$F_{6,\ 245} = 1.12$
	$^*p = 0.010$	$p = 0.82$	$p = 0.32$	$p = 0.85$	$p = 0.35$

To check the robustness of results with respect to deviations from homoscedasticity and normality we repeated the analysis using Box–Cox transformed data (details not shown).
*refers to $p < 0.05$ on Box–Cox transformed data.

however, effects of mutant versus wildtype alleles differed only for two traits, onset of reproduction ($F_{1,8} = 10.20$, $p = 0.01$) and late fecundity ($F_{1,8} = 6.22$, $p = 0.034$). The results confirmed our expectation that differences between mutant and wildtype alleles may be more pronounced in tester than in control crosses.

Effects of transgene versus wildtype alleles

(1) For none of the traits did the average trait value differ between the tester and the control cross (details of ANOVA not shown, cf. Figure 1; cross effect, in all cases: $F_{1,\ 2} < 7.5$, $p > 0.10$), thus confirming our expectation that the transgene provides a functional rescue.

(2) The interaction contrast comparing the transgene to the average of the 3 wildtype alleles was only significant for early fecundity (details of ANOVA not shown, cf. Figure 1; $F_{1,\ 8} = 8.78$, $p = 0.018$); for other traits the contrasts were non-significant ($F_{1,\ 8} < 4.0$, $p > 0.08$), confirming that allelic effects did not differ between the transgene and wildtype alleles for most traits.

(3) When separately analyzing tester and control crosses, there were for most traits no significant differences between the effects of the transgene versus wildtype alleles (details of ANOVA not shown, cf. Figure 1; contrast: $F_{1,3} < 7.8$, $p > 0.05$). Specifically, when analyzing tester crosses, we found significant 'transgene-wildtype' contrasts for developmental time ($F_{1,\ 3} = 12.61$, $p = 0.023$), late fecundity ($F_{1,3} = 12.96$, $p = 0.022$) and total fecundity ($F_{1,3} = 7.8$, $p = 0.049$), but no differences in the effects between the transgene and the wildtype alleles for early fecundity and the onset of reproduction. When analyzing control crosses, only early fecundity differed significantly between the transgene and the wildtype ($F_{1,3} = 12.01$, $p = 0.026$), whereas developmental time, onset of reproduction, late and total fecundity did not differ. The results therefore suggest that, for 2 out of traits in the tester and 4 out of 5 traits in the control cross, the transgenic line provides a rescue of MET function, and that allelic effects do typically not differ between the transgene and the wildtype alleles.

Allelic effects on trait relationships

(1) We found significant effects of AB_{ij} interactions on the linear relationships between traits (covariate × AB_{ij} interaction tests, Table 4), indicating that alleles differed in their effects on phenotypic covariances. Specifically, we detected allelic variation for the effects on the linear relationships between developmental time and onset of reproduction, onset of reproduction and early fecundity, onset of reproduction and late fecundity, and early fecundity and late fecundity. In contrast, slopes were homogeneous for the relationships between developmental time with both early and late fecundity, and we were thus able to test for allelic variation while controlling for the covariate. In both cases, allelic variation did not affect fecundity when variation in developmental time was taken into account (AB_{ij} interaction test, Table 4). Thus, in summary, the ANCOVA revealed that alleles typically differed in their effects on phenotypic covariances, rather than affecting single traits independent of other traits, thereby again confirming life history pleiotropy at the *Met* locus.

(2) With the exception of a single case we did not find any significant intralocus genetic correlation. This may have two reasons. First, the variable allelic effects on phenotypic covariances, as detected in the ANCOVA, suggest that most alleles vary in the signs and magnitudes of their pleiotropic effects. Consequently, pleiotropic effects may on average cancel out, leading to a net genetic correlation close to zero. Second, statistical power was low ($n = 10$ variable alleles). Allelic effects for developmental time and onset of reproduction did not covary ($r = 0.14$, $p = 0.71$). Yet, developmental time and early fecundity were negatively correlated (Figure 2, $r = -0.84$, $p = 0.0024$), suggesting that *Met* alleles have consistent pleiotropic effects on these traits. Thus, alleles had on average significantly positive (++, −−) pleiotropic effects on fitness, i.e. alleles that decreased developmental time increased fecundity and alleles that increased developmental time decreased fecundity. Consequently, contrary to our expectation, the intralocus correlation did not suggest a contribution of the *Met* locus to a polygenic trade-off between these traits. We also

Table 4. Results of ANCOVAs on onset of reproduction, early and late fecundity

Source	Covariate	Onset of reprod.	Fecundity (1–10 days)		Fecundity (10–20 days)		
		X	*X*	*Y*	*X*	*Y*	*Z*
Allele (A_i)		$F_{9,\ 797.5} = 1.3$	$F_{9,\ 790.3} = 1.3$	$F_{9,\ 363.6} = 4.2$	$F_{9,\ 689.5} = 0.9$	$F_{9,\ 453.5} = 3.3$	$F_{9,\ 667.8} = 1.0$
		$^*p = 0.21$	$p = 0.23$	$^*p < 0.0001$	$p = 0.51$	$^*p = 0.0006$	$p = 0.44$
Cross (B_j)		$F_{1,\ 797.6} = 5.1$	$F_{1,\ 789.7} = 1.9$	$F_{1,\ 463.4} = 1.2$	$F_{1,\ 689} = 0.03$	$F_{1,\ 527.4} = 0.01$	$F_{1,\ 677.1} = 0.4$
		$^*p = 0.025$	$p = 0.16$	$p = 0.28$	$p = 0.86$	$p = 0.92$	$p = 0.54$
AB_{ij}		$F_{9,\ 797.5} = 3.3$	$F_{9,\ 790.3} = 0.5$	$F_{9,\ 363.6} = 6.4$	$F_{9,\ 698.5} = 0.9$	$F_{9,\ 453.5} = 2.1$	$F_{9,\ 667.8} = 2.2$
		$p = 0.0005$	$p < 0.89$	$^*p < 0.0001$	$p = 0.56$	$^*p = 0.032$	$p = 0.024$
Vial (AB_{ij})		$F_{20,\ 790} = 3.4$	$F_{20,\ 788} = 0.9$	$F_{20,\ 787} = 0.7$	$F_{20,\ 688} = 0.7$	$F_{20,\ 687} = 0.7$	$F_{20,\ 688} = 0.7$
		$p < 0.0001$	$p = 0.50$	$p = 0.79$	$p = 0.80$	$p = 0.78$	$p = 0.78$
Covariate (c)		$F_{1,\ 790} = 0.0004$	$F_{1,\ 788} = 0.4$	$F_{1,\ 787} = 17.2$	$F_{1,\ 688} = 2.4$	$F_{1,\ 687} = 0.1$	$F_{1,\ 688} = 0.6$
		$^*p = 0.98$	$p = 0.54$	$^*p < 0.0001$	$p = 0.12$	$p = 0.74$	$p = 0.45$
$c \times A_i$		$F_{9,\ 790} = 1.5$	$F_{9,\ 788} = 1.2$	$F_{9,\ 787} = 1.5$	$F_{9,\ 688} = 1.0$	$F_{9,\ 687} = 1.5$	$F_{1,\ 688} = 1.0$
		$^*p = 0.14$	$p = 0.32$	$p = 0.16$	$p = 0.47$	$^*p = 0.13$	$p = 0.41$
$c \times B_j$		$F_{1,\ 790} = 4.9$	$F_{1,\ 788} = 2.2$	$F_{1,\ 787} = 0.01$	$F_{1,\ 688} = 0.04$	$F_{1,\ 687} = 1.0$	$F_{1,\ 688} = 1.5$
		$^*p = 0.027$	$p = 0.14$	$p = 0.91$	$p = 0.83$	$p = 0.31$	$^*P = 0.22$
$c \times AB_{ij}$		$F_{9,\ 790} = 3.4$	$F_{9,\ 788} = 0.5$	$F_{9,\ 787} = 1.9$	$F_{9,\ 688} = 0.8$	$F_{9,\ 687} = 2.4$	$F_{9,\ 688} = 2.1$
		$p = 0.0004$	$p = 0.89$	$^*p = 0.048$	$p = 0.58$	$^*p = 0.012$	$p = 0.025$

The assumption of homogeneity of slopes was tested as an interaction between the main effects (or interactions) and the covariate. To check the robustness of results with respect to deviations from homoscedasticity and normality we repeated the analysis using Box–Cox transformed data (details not shown).

Abbreviations: *X* = developmental time, *Y* = onset of reproduction, *Z* = fecundity (1–10d).

*refers to $p < 0.05$ on Box–Cox transformed data.

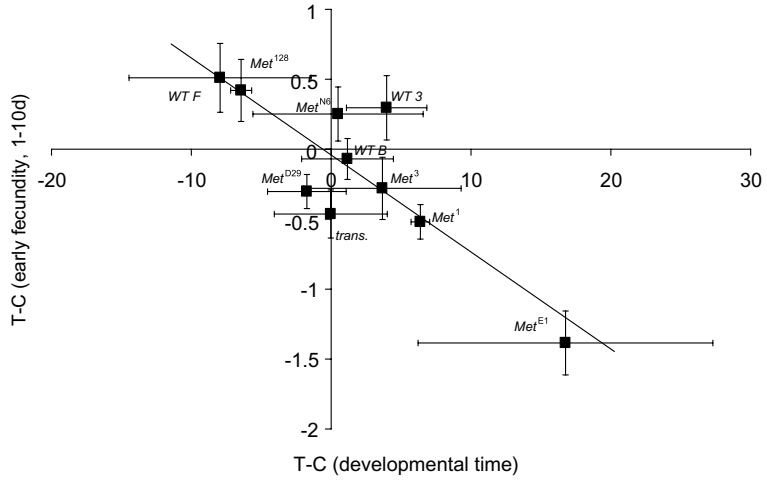

Figure 2. Intralocus genetic correlation between developmental time and early fecundity over the first 10 days. Data shown are untransformed bivariate $t-c$ differences between means (± 1 SE).

note that, although the correlation is based on only 10 alleles and the Met^{E1} allele seems to have a strong effect on the correlation, the negative relationship seems to be robust (e.g., insensitive to outliers) as confirmed by a significant nonparametric correlation (Spearman's rank correlation; $r_s = -0.64$, $p = 0.048$). The $t-c$ values for developmental time and late fecundity, however, were not correlated ($r = 0.18$, $p = 0.61$) Similarly, the onset of reproduction was not correlated with early fecundity ($r = -0.38$, $p = 0.27$) and late fecundity ($r = 0.53$, $p = 0.11$). Finally, early and late fecundity ($r = -0.24$, $p = 0.50$) were also not correlated.

Discussion

Allelic variation at loci coding for hormone receptors or binding proteins may underlie phenotypic variation and covariation between life history traits (e.g., Zera & Harshman, 2001). Specifally, we may expect that loci associated with hormone metabolism are pleiotropic because alleles at such loci affect the regulation of hormones, which, in turn, have multiple phenotypic effects (Rose, 1991; Finch & Rose, 1995; Sinervo & Svensson, 1998). Using a candidate gene approach, we have confirmed this hypothesis by showing that alleles of *Met*, a locus encoding a putative JH receptor, vary in their effects on both uni- and multivariate life history traits. Thus, the allelic effects of *Met* support genetic models where pleiotropy at genes associated with hormone regulation can contribute to the evolution of life history traits.

Allelic effects on univariate traits

Our main finding is that allelic variation at *Met* affects multiple life history traits. First, we found that *Met* alleles differed significantly in their effects on the onset of reproduction. As can be seen from Figure 1, the difference between the tester and control cross (t–c), a measure of the heterozygous allelic effect, depends on the allele and is either positive ($t > c$), negative ($t < c$), or zero ($t = c$). This suggests that some alleles delay the onset of reproduction, others shorten it, and still others do not affect this trait.

Although we have not measured JH titers or JH binding affinities of the MET proteins for the *Met* strains used in our experiment, evidence suggests that alleles of *Met* specifically differ in their binding affinities for JH, but not in other aspects of JH metabolism (Shemshedini & Wilson, 1990; Shemshedini, Lanoue & Wilson, 1990). Consequently, we believe that the phenotypic variation and covariation among *Met* alleles observed in our experiment may, at least partially, be explained by variation in binding affinity of MET for juvenile hormone. Variation in binding affinities may have

similar phenotypic consequences as variation in JH signaling, for example as differences in juvenile hormone titers. Clearly, this remains to be tested in future work. Yet, our interpretation of the results is consistent with physiological models of JH action.

For instance, Manning (1967) found that adult *D. melanogaster* show an earlier onset of reproduction when JH-producing corpora allata are injected at the pupal stage, suggesting that JH regulates the onset of reproduction. Similarly, it has been suggested that the early sexual maturation of the *aa* (*abnormal abdomen*, ur^{aa}) genotype of Hawaiian *Drosophila mercatorum* is due to reduced JHE activity, leading to an unusually high JH titer (Templeton & Rankin, 1978; DeSalle & Templeton, 1986). Recent evidence also indicates that JH regulates the onset and termination of reproductive diapause in *D. melanogaster* (Tatar & Yin, 2001; Tatar, Chien & Priest, 2001). Most interestingly, however, Wilson and Ashok (1998) have found that females homozygous for the *Met* null allele ($wv\ Met^{27}$) genotype lag behind wildtype females for the onset of oviposition. This defect can be rescued by a transgene carrying an ectopic wildtype allele ($wv\ Met^{27}$; $p[w^+\ EN71]/+$), suggesting that the effect on the onset of oviposition is specific to the *Met* locus. Thus, our study confirms that allelic variation at *Met* can affect the onset of reproduction, presumably through genetically caused variation in JH metabolism. In particular, we have shown that wildtype alleles show detectable allelic variation for this trait.

Second, quantitative complementation revealed that *Met* alleles differ in their effects on early, late, and total fecundity. Again, some alleles seemed to decrease components of age-specific fecundity while others seemed to increase them (cf. Figure 1). Given that JH (or methoprene) regulates oogenesis by stimulating vitellogenesis (e.g., Wilson, Landers & Happ, 1983; Salmon, Marx & Harshman, 2001), allelic variation for JH-binding is expected to lead to variation in fecundity. In particular, our results are consistent with previous work by Minkoff and Wilson (1992) and Wilson and Ashok (1998), showing that *Met* specifically affects early fecundity. For instance, Wilson and Ashok (1998) demonstrated that the strong ovipositional defect observed in $wv\ Met^{27}$ null mutants can be specifically rescued using the $wv\ Met^{27}$; $p[w^+\ EN71]/+$ transgene. This ovipositional defect has been shown to be caused by a reduced number of vitellogenic oocytes (Wilson & Ashok, 1998), consistent with the role of JH in vitellogenic oocyte development (see Jowett & Postlethwaith, 1980; Bownes, 1982; Wilson, 1982; Wilson, Landers & Happ, 1983; Soller, Bownes & Kubli, 1999). Here we have shown that, for a broad range of alleles, variation at *Met* not only affects early fecundity, but also late fecundity, suggesting that the effects of the *Met* locus are not restricted to early life. Thus, *Met* is clearly implied in having specific effects on several age-specific components of fecundity, probably because of its role in JH metabolism.

The allelic effects in our experiment were rather small. Since QCT cannot be used to conclusively distinguish between (i) epistasis between an unknown genetic factor and *Met* and (ii) allelism at *Met* (see Long et al., 1996; Mackay & Fry, 1996; Gurganus et al., 1999; Palsson & Gibson, 2000), our results suggest that either the indirect (epistatic) and/or the direct (allelic) effects of *Met* are small. Thus, MET may be only marginally involved in JH signaling, in agreement with the idea that another gene codes for the JH receptor. Alternatively, there may exist some kind of functional redundancy in terms of JH signaling, where other components than *Met* can mediate JH signals. Although the various components of JH signaling are not yet satisfactorily worked out in *Drosophila*, there exist several candidate genes for the JH receptor (also see below), various genes coding for JH binding proteins, and a handful of examples of JH inducible genes (e.g., Gilbert, Rybczynski & Tobe, 1996; Riddiford, 1996; Dubrovsky et al., 2000; Gilbert, Granger & Roe, 2000; Truman & Riddiford, 2002). Furthermore, *D. melanogaster* and other higher dipterans have two forms of JH (JH III and JH III bisepoxide) which may require different receptors or binding proteins. It also remains the possibility that different tissues or developmental stages rely on different JH receptors or binding proteins.

Allelic effects on trait relationships

We also found more direct evidence for pleiotropy at *Met* by investigating allelic effects on trait relationships. In particular, we found that *Met* alleles differed in their effects on phenotypic covariances between several traits (developmental time and

onset of reproduction; onset of reproduction and both early and late fecundity; early and late fecundity). This suggests the existence of allelic variation for pleiotropic effects, i.e., variation in the signs and magnitudes of pleiotropic effects, so that different alleles have different effects on bivariate (or multivariate) phenotypes. Such variation within (and among) loci for the details of pleiotropic gene action is evolutionarily interesting because it implies that pleiotropic effects and thus genetic architecture can evolve (e.g., Cheverud, 2001). Although the evolutionary significance of pleiotropy was recognized relatively early (e.g., Caspari, 1952; Williams, 1957; Wright, 1968; Rose, 1985), genetic variation for pleiotropy has only rarely been studied (e.g., see Gromko, 1995; Lyman & Mackay, 1998; Cheverud, 2001). Clearly, future studies are required that quantify allelic variation for pleiotropy and investigate the evolutionary significance of such variation.

We also investigated genetic covariance among traits induced by *Met* alleles. When considering the effects of alleles on two traits, allelic effects were in most cases not correlated. On the one hand, it is quite likely that this failure was due to low power ($n = 10$). On the other hand, the absence of correlations may indicate that the magnitudes and signs of allelic effects were not consistent among alleles, although some individual alleles clearly had pleiotropic effects on the two traits (cf. Figure 1). This may illustrate the important – yet rarely appreciated – principle that a lack of genetic correlation does not imply a lack of pleiotropy (e.g., Gromko, 1995; Lyman & Mackay, 1998). Thus, given genetic variation in the signs and magnitudes of allelic effects, pleiotropic effects may cancel out, resulting in a net correlation of zero.

However, despite the low power in detecting correlations, we found that pleiotropic effects on developmental time and early fecundity are negatively correlated. Thus, *Met* alleles that decrease developmental time increase fecundity, while alleles that increase developmental time decrease fecundity. Typically, decreasing developmental time while increasing fecundity increases fitness, whereas increasing developmental time while decreasing fecundity reduces fitness. Our finding therefore suggests that alleles had on average significantly positive ($++$, $--$) pleiotropic effects on the two fitness components. In contrast, quantitative genetic and selection experiments have found a positive polygenic genetic correlation between these two traits, suggesting a fitness trade-off due to negative (antagonistic, $+-$, $-+$) pleiotropic effects (e.g., Zwaan, Bijlsma & Hoekstra, 1995). Direct estimates of pleiotropic effects underlying genetic correlations have rarely been undertaken and the exact nature of the pleiotropy may be complex (e.g., Houle, 1991, 1998; Martorell, Toro & Gallego, 1998). For instance, in contrast to conventional wisdom, negative genetic covariances between fitness components are not necessarily indicative of underlying life history constraints or functional trade-offs (e.g., Houle, 1991). Furthermore, mutational effects of *de novo* mutations do typically not show negative (antagonistic) pleiotropy, but exhibit positive pleiotropic ($++$, $--$) effects on fitness components (e.g., Houle et al., 1994). Such positive pleiotropy seems typically to be caused by mutations with deleterious pleiotropic effects (e.g., Martorell, Toro & Gallego, 1998). Thus, our results, which are partially based on mutant alleles isolated from mutant screens, may be consistent with the finding that *de novo* mutations typically show positive pleiotropy for fitness (e.g., Houle et al., 1994).

Although we have found some preliminary evidence that *Met* mutant alleles may have partially recessive and deleterious effects on fitness, we believe it to be unlikely that the negative correlation is entirely due to deleterious pleiotropic effects of mutant as compared to wildtype alleles. First, there was variation in both the sign and magnitude of allelic effects both among mutant and wildtype alleles (Figures 1 and 2). Second, for most traits, the phenotypic effects attributable to allelic variation did typically not differ between mutant and wildtype alleles as revealed by the nonsignificant 'mutant-wildtype' interaction contrasts for the AB_{ij} interactions. Third, the existence of a polygenic trade-off between two fitness components does not imply that all loci affecting these characters will show a negative genetic covariance of allelic effects on fitness components. Since polygenic correlations are statistical measures that average over positive and negative (antagonistic) pleiotropic effects of many alleles and loci (e.g., Gromko, 1995; Lyman & Mackay, 1998), it may be that most *Met* alleles have deleterious effects on these fitness components that are overcompensated by antagonistic effects at other loci. In summary, although our finding of positive pleiot-

ropy at the *Met* locus does not support the antagonistic pleiotropy (trade-off) model of evolutionary genetics, it also does not seriously challenge it (cf. discussion in Houle et al., 1994; Martorell, Toro & Gallego, 1998).

As suggested by Hudak and Gromko (1989) and Zwaan, Bijlsma and Hoekstra (1995), the positive genetic correlation between developmental time and early fecundity observed in many experiments may be the consequence of the multiple phenotypic effects of JH ('hormonal pleiotropy', Finch & Rose, 1995). An increased JH titer would prolong developmental time and stimulate early fecundity. This hypothesis is, however, very difficult to evaluate because the molecular and physiological effects of JH on developmental time are still very poorly understood. Yet, although our results do not support the hypothesis suggested by Hudak and Gromko (1989) and Zwaan, Bijlsma and Hoekstra (1995), our finding of a negative intralocus genetic correlation at *Met* clearly needs to be validated by studying more *Met* alleles. The correlation we have detected is based on only 10 alleles, and we also note that, although the rank correlation we have estimated is insensitive to outliers, the Met^{E1} allele seems to have a strong effect on the correlation.

Molecular nature of Met

Met belongs to the basic helix-loop-helix (bHLH)-PER-AHR/ARNT-SIM (PAS) family of transcription factors (Ashok, Turner & Wilson, 1998), and is similar to *tango*, the *Drosophila* ARNT homolog, which, in vertebrates, forms a heterodimer with the aryl hydrocarbon receptor (AHR), involved in binding xenobiotic toxicants such as dioxin. Although this gene structure is consistent with previous findings of JH effects on gene expression (Jones, 1995), there remain uncertainties surrounding the function of *Met* (e.g., Truman & Riddiford, 2002). For example, it is currently unclear whether MET represents a JH receptor or another form of JH binding protein, whether it forms dimers with other proteins involved in endocrine signaling, and to which target genes it signals.

However, all the evidence at hand suggests that *Met* is specifically involved in high-affinity juvenile hormone binding or reception (e.g., Wilson & Fabian, 1986; Shemshedini & Wilson, 1990; Shemshedini, Lanoue & Wilson, 1990; Ashok, Turner & Wilson, 1998; Restifo & Wilson, 1998; Wilson & Ashok, 1998; Pursley, Ashok & Wilson, 2000). For example, while *Met* does not seem to affect penetration, excretion, tissue sequestration, and metabolism of JH (see Shemshedini & Wilson, 1990), *Met* genotypes specifically differ in their binding affinities for JH (Shemshedini & Wilson, 1990; Shemshedini, Lanoue & Wilson, 1990). Furthermore, the potential significance of *Met* in endocrine signaling is supported by the observation that *Met* interacts with other loci affecting hormone metabolism. For instance, the $Fas2^{spin}$ mutant allele of the *fasciclin* gene affects the looping and rotation of genitalia, stimulates the overproduction of JH (i.e., is allatotropic), and interacts epistatically with mutations at *Met* (Ádám, Perrimon & Noselli, 2003). Similarly, the *Broad* gene, a major ecdysone-inducible developmental gene required during metamorphosis, appears to interact with *Met* (Restifo & Wilson, 1998).

Although our results are consistent with both physiological models of JH action and the suggestion that the *Met* locus may code for a JH receptor, *Met* null mutants are viable and show no apparent defects in embryogenesis or larval development – as may be expected if MET is a JH receptor (Wilson & Ashok, 1998). In fact, recent evidence suggests the *ultraspiracle gene* (usp) as another candidate coding for the JH receptor (cf. Truman & Riddford, 2002). Yet, in contrast to MET, the USP protein does not fulfill the criterion of high-affinity hormone binding which is typically required for a hormone receptor. Thus, at present, the exact molecular nature of the JH receptor remains elusive (e.g., Gilbert, Granger & Roe, 2000; Truman & Riddiford, 2002; for an alternative view to the classical model postulating the existence of a JH receptor see Wheeler & Nijhout, 2003). Nevertheless, the *Met* locus appears to be intimately involved in JH signaling (e.g., Ashok, Turner & Wilson, 1998; Restifo & Wilson, 1998; Pursley, Ashok & Wilson, 2000; Ádám, Perrimon & Noselli, 2003), and our results are clearly consistent with multiple life history effects of JH mediated by MET.

Conclusions

Here we have provided preliminary evidence that a putative hormone receptor gene has pleiotropic effects on life history traits, presumably through

genetic variation in JH binding. Our data thus support genetic models where pleiotropy at genes associated with hormone regulation can contribute to the evolution of life history traits.

From a methodological point of view, our study illustrates that, using the QCT method, one can detect life history pleiotropy at a single locus and study whether and how pleiotropic effects are correlated (see Leroi, 2001). However, the QCT method does not provide a perfect control for the effects of genetic background. Thus, our results need to be validated in future studies, for example by using overexpression of particular mutant alleles in maximally controlled genetic backgrounds. Yet, in practically all cases, a significant QCT result suggests that the candidate gene has effects on the traits studied, either through epistasis or through allelism or both (Long et al., 1996; Mackay & Fry, 1996; Gurganus et al., 1999; Palsson & Gibson, 2000).

The analysis of specific loci affecting life history traits is of central importance to the testing of the assumptions of life history theory and evolutionary genetics with regard to genetic architecture (e.g., Houle, 1991; Stearns & Kaiser, 1996; Johnson & Shook, 1997; Leroi, 2001; Knight, Azevedo & Leroi, 2001). By studying the effects of endocrine loci on life history traits one may establish causal links between genes, hormonal mechanisms, fitness components, and trade-offs.

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Appendix Sample sizes for life history trait measurements (see text for definitions) in the tester (*t*) and control crosses (*c*) for each test allele. *trans.* = transgene, *WT* = wildtype

Cross	Develop. time	Onset of reprod.	Fec. (1–10 days)	Fec. (10–20 days)	Fec. total
Met^{1} *t*	49	48	48	44	48
Met^{1} *c*	55	48	49	42	49
Met^{128} *t*	35	31	31	26	31
Met^{128} *c*	63	50	50	45	50
Met^{3} *t*	63	30	28	22	29
Met^{3} *c*	48	33	32	24	33
Met^{E1} *t*	80	50	50	45	50
Met^{E1} *c*	35	33	33	32	33
Met^{N6} *t*	53	50	50	40	50
Met^{N6} *c*	67	47	47	41	47
Met^{D29} *t*	47	42	42	28	42
Met^{D29} *c*	62	39	39	34	39
trans. t	56	46	46	41	46
trans. c	49	46	46	44	46
WT B t	54	39	39	36	39
WT B c	48	42	42	40	42
WT F t	43	39	39	37	39
WT F c	71	49	49	44	49
WT 3 t	38	38	38	34	38
WT 3 c	58	50	50	49	50

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