LETTER

Differential effects of genetic vs. environmental quality in *Drosophila melanogaster* suggest multiple forms of condition dependence

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Abstract

Condition is a central concept in evolutionary ecology, but the roles of genetic and environmental quality in condition-dependent trait expression remain poorly understood. Theory suggests that condition integrates genetic, epigenetic and somatic factors, and therefore predicts alignment between the phenotypic effects of genetic and environmental quality. To test this key prediction, we manipulated both genetic (mutational) and environmental (dietary) quality in *Drosophila melanogaster* and examined responses in morphological and chemical (cuticular hydrocarbon, CHC) traits in both sexes. While the phenotypic effects of diet were consistent among genotypes, effects of mutation load varied in magnitude and direction. Average effects of diet and mutation were aligned for most morphological traits, but non-aligned for the male sexcombs and CHCs in both sexes. Our results suggest the existence of distinct forms of condition dependence, one integrating both genetic and environmental effects and the other purely environmental. We propose a model to account for these observations.

Keywords

Condition-dependent, diet, genic capture, good genes, mutation load, sexual selection.

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INTRODUCTION

For many fitness-related traits, increased phenotypic expression enhances mating success or fecundity, but these positive effects come at the cost of reduced viability. Such trade-offs are believed to characterise many sexually selected traits (Cotton *et al.* 2004), as well as fecundity-related structures such as ovaries (e.g., Zera *et al.* 1998). Theory suggests that traits subject to such trade-offs should evolve heightened condition dependence: a developmental mechanism that links trait expression to individual condition and thereby allows individuals to express these traits to the maximum degree that they can afford (Andersson 1982; Nur & Hasson 1984; Houle 1991). Condition has been variously defined as the availability and processing efficiency of metabolic resources (Rowe & Houle 1996) or, more broadly, as the cellular capacity to withstand environmental challenges (Hill 2011).

It is widely assumed that the expression of condition-dependent traits should reflect genetic quality (i.e., the presence of high-fitness alleles) at many loci (Andersson 1982), and the 'genic capture' model applies this to the evolution of costly sexually selected traits in particular, predicting that expression of such traits should provide a sufficiently large mutational target to reflect genetic variation in fitness (Rowe & Houle 1996). As a consequence, the condition dependence of male sexual performance may underlie the maintenance of costly

female mate preferences (Andersson 1982; Rowe & Houle 1996), and may also contribute to the purging of deleterious mutations (Whitlock & Agrawal 2009) and to the evolution of sex (Agrawal 2001; Siller 2001). Yet, while genic capture and many signaller–receiver coevolution models focus on the effects of 'good genes' (Rowe & Houle 1996; Tomkins *et al.* 2004; Birkhead *et al.* 2006), condition is also likely to be strongly influenced by environmental effects (Hill 2011), and both of the above definitions of condition explicitly include environmental as well as genetic contributors. Indeed, empirical investigation of condition dependence has largely relied on measures of the sensitivity of trait expression to changes in environmental quality (Cotton *et al.* 2004), likely because it is easier to manipulate than genetic quality. However, very few studies have attempted direct comparisons of the effects of environmental vs. genetic quality on trait expression, and it is therefore unclear how evidence of environmental sensitivity relates to a trait's response to mutation load and hence its capacity to signal 'good genes' (Tomkins *et al.* 2004).

Condition-dependence theory (Andersson 1982; Nur & Hasson 1984; Houle 1991; Rowe & Houle 1996; Hill 2011) is widely interpreted as predicting that the effects of environmental and genetic quality on trait expression should be aligned (i.e. similar in direction) because both are mediated through condition. An adverse environment (e.g. nutritional stress) and high load of (primarily deleterious) mutations

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should both act to depress condition and thereby reduce the expression of condition-dependent traits (Fig. 1a). It is therefore assumed that traits that exhibit heightened sensitivity to environmental quality can nonetheless serve as honest signals of genetic quality (Andersson 1982; Hunt *et al.* 2004; Tomkins *et al.* 2004). However, this alignment assumption has not been tested (Whitlock & Agrawal 2009) and may not always hold. In particular, it is possible that the expression of some condition-dependent traits is affected by genotype at just one or a few loci that determine the ability to acquire and process particular micro-nutrients involved in specific biochemical pathways. Such traits may be sensitive to at least some aspects of environmental quality (e.g., factors that affect availability of the relevant micro-nutrients), but their expression would provide a poor signal of genome-wide genetic quality (see Houle 1991; Schielzeth *et al.* 2012). In addition, for some traits the set of loci regulating resource allocation may constitute a relatively large mutational target and mutations at these loci could act to increase or decrease the expression of a trait, largely independent of condition, by altering relative allocation among traits.

We investigated the effects of genetic and environmental quality by manipulating mutation load and nutrient concen-
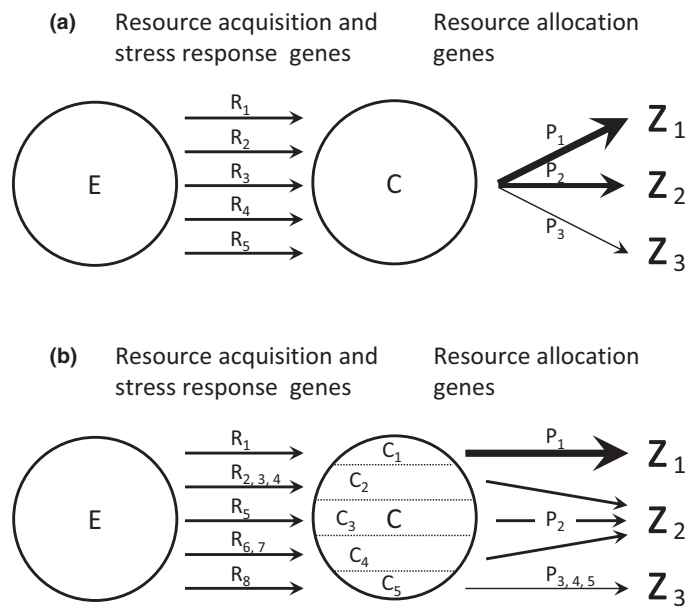

Figure 1 (a) A simplified, synthetic representation of a widely accepted model of condition-dependent trait expression (based on Andersson 1982; Houle 1991; Rowe & Houle 1996; Hill 2011). Ability to acquire and assimilate relevant environmental resources (E) that contribute to the functionality of vital cellular processes, controlled by genes R_n, determines an individual's condition (C). Phenotypic traits Z_n exhibit differential condition dependence, represented by thickness of arrows from C, according to a resource allocation pattern determined by genes P_n. (b) A modified model whereby expression of some traits is limited by availability of particular micro-resources, or ability to overcome specific environmental stressors (C_n), which is influenced by a subset of R_n. For example, expression of Z_1 may be sensitive to environmental quality (which determines availability of C_1) but relatively insensitive to overall genetic quality, whereas expression of Z_2 may be sensitive to both environmental and genetic quality.

tration in the larval diet in a factorial design replicated across 19 haploid genomes ('hemiclonal lines': Abbott & Morrow 2011) randomly selected from an outbred, laboratory-adapted population of *Drosophila melanogaster*. We examined treatment effects on several different types of traits that are expected to exhibit heightened condition dependence: (1) morphological traits that function in sexual signalling in *D. melanogaster* males, including the sexcomb, a group of modified bristles on the foreleg which is thought to be used in tactile signalling during courtship and mating (Ahuja & Singh 2008; Ng & Kopp 2008; Ahuja *et al.* 2011), and the wing, which is vibrated to produce the 'courtship song' (Abbott *et al.* 2010; Menezes *et al.* 2013), (2) cuticular hydrocarbons and their derivatives bearing various molecular functional groups (henceforth CHCs), a complex blend of compounds on the cuticle that can be assessed by other individuals and functions as a chemical signal during courtship (Jallon 1984; Savarit *et al.* 1999; Rybak *et al.* 2002; Ferveur 2005; Grillet *et al.* 2006; Ejima *et al.* 2007; Yew *et al.* 2009), (3) male body size, which is subject to positive sexual selection in some competitive environments (Pavković-Lučić *et al.* 2009; Pavković-Lučić & Kekić 2011), (4) female body size, which is strongly associated with egg production and therefore subject to fecundity selection (Berglund *et al.* 2008). For comparison, we also examined treatment effects on traits that appear to be less strongly linked to fitness and are therefore not expected to exhibit heightened condition dependence, including non-sexual male morphological traits (head and foreleg dimensions), female morphological traits (head, foreleg and wing dimensions) and female CHCs (which confer desiccation resistance in the natural environment, but are likely to be subject to weak viability selection under benign, and relatively humid, laboratory conditions (Kwan & Rundle 2010)).

This experiment allowed us to test several assumptions that underpin the interpretation of many empirical studies on condition dependence: (1) Genetic and environmental quality should align in their effects on trait expression (with both high mutation load and low-quality diet consistently reducing trait expression across genotypes), because the effects of both environmental and genetic quality are mediated through condition. (2) Traits closely associated with fitness (male secondary sexual traits, female body size) should exhibit heightened condition dependence, reflected in high sensitivity of expression to both environmental and genetic quality. (3) Conversely, traits presumed to be weakly linked to fitness (non-sexual morphological and CHC traits) should exhibit low average sensitivity to environmental and genetic quality. Instead, such traits should exhibit genotype-specific developmental responses, whereby high mutation load can result in either reduced or enhanced trait expression, because mutational effects are not mediated through condition.

MATERIAL AND METHODS

Mutation treatment

A random sample of 19 haploid genomes (X + autosomes; henceforth 'haplotypes') was obtained from the outbred, laboratory-adapted *Ives* (*IV*) population using the *Drosophila*

hemiclone system (Abbott & Morrow 2011), as described in Mallet *et al.* (2012). Hemiclones are individuals that possess identical copies of a particular haploid genome (haplotype), and sets of genetically distinct hemiclones can be used to investigate haplotype effects (a combination of additive-genetic and epistatic effects) on phenotype and fitness. Each hemiclone line was used to found both a mutation accumulation (MA) and a control (C) line. MA lines were propagated, without recombination, via a single haploid genome every generation, and the near-absence of selection facilitated the accumulation of novel mutations. Controls were maintained at a population size of 16–25 males/generation, without recombination, allowing selection via clonal competition among haploid genomes, thus reducing the opportunity for MA. After 50 generations under these propagation regimes, experimental flies were generated by expressing the C and MA haplotypes alongside random haplotypes from the *IV* population as described in Kimber & Chippindale (2013). Previous studies on these lines confirmed that the MA haplotypes, when thus expressed in combination with random haplotypes from the outbred population, resulted in significant declines relative to control lines in juvenile viability (31% decline), male mating/fertilisation success (54% decline) and the X-linked component of adult female fitness (7% decline) (Mallet *et al.* 2011, 2012). The MA haplotypes thus reduced fitness even in individuals that were heterozygous at many loci.

Diet treatment

A preliminary experiment showed that a 70% dilution of the standard food medium had little effect on development and no effect on larval viability, and thus on opportunity for viability selection (Fig. S1). Flies from all 19 C and MA lines were therefore raised for one generation on both the 100% ('high-quality') and 70% ('low-quality') media. At least five replicate vials were created for each line × mutation × diet × sex combination. Offspring were mixed among these vials and then an average of 9.4 (range 7–12) random virgin individuals of the relevant sex were collected and stored in same-sex groups of ten flies/vial for 1–3 days prior to CHC extraction. In total, 1444 individuals were phenotyped.

Phenotyping

CHCs were extracted and analysed on an Agilent Technologies (Wilmington, DE, USA) 6890N gas chromatograph as described in Kwan & Rundle (2010). Individual CHC profiles were determined by the integration of the area under 34 peaks in females and 24 peaks in males (Fig. S2). CHC values were converted to relative proportions by dividing the area under each peak by total area under all peaks for a given individual to correct for technical error associated with estimating absolute concentrations. From these data, centred log ratios (CLRs) were computed for each peak (see Supporting Information), and these CLR-transformed values were used in subsequent analyses.

Following CHC extraction, the flies were preserved in 70% ethanol. Subsequently, each individual was placed into a droplet of glycerol on a microscope slide and imaged from the side using a DFC420 camera mounted on a Leica MZ16A computer-operated stereoscope (Wetzlar, Germany). The head, one wing and one foreleg were then removed using micro-shears and micro-needle probes and re-imaged separately. From these images, measurements of thorax length, head length, head height, head width, fore-tibia length, wing length (length of the R_{4+5} vein from the r-m cross vein to the wing margin) and (in males) sexcomb row width (Fig. S3) were made in mm using ImageJ software (Schneider *et al.* 2012). Repeatabilities were moderate to high for all traits (range: 0.62–0.94; Table S1).

Statistical analyses

Treatment effects on morphological traits were tested in several ways. First, we analysed treatment effects on standardized individual scores for the first six principal components (PCs) from principal component analysis on the morphological trait correlation matrix (Tables S2, S3). Scores on PC1 can be interpreted as 'body size', given that all traits loaded in the same direction on this PC in both sexes. The other PCs can be interpreted as shape traits. Second, we tested effects on standardised raw measurements (i.e. z-scores: mean = 0, variance = 1) for each of the separate morphological traits in each sex. Third, for comparison, we investigated treatment effects on standardised morphological traits in analysis of covariance (ANCOVA) models with thorax length included as a covariate in order to examine variation in trait size while partially controlling for variation in body size. ANCOVA results (described in the Supporting Information) are broadly consistent with those from the other analyses outlined above.

Treatment effects on CHCs were investigated using unstandardized individual scores on the first six principal components of the covariance matrix of CLR-transformed traits in each sex (Table S4, S5). We did not analyse separate CHCs because the diversity of compounds involved, strong covariances among them, and the complex nature of the chemical communications in which they participate make it problematic to infer the signalling role of specific compounds or particular combinations of them (Everaerts *et al.* 2010).

For each trait/principal component, we employed a separate general linear mixed model, fit separately within each sex via restricted maximum likelihood (REML) with unbounded variance components:

$$\text{trait} = M + D + M \times D + \text{Line} + M \times \text{Line} + D \times \text{Line} + M \times D \times \text{Line}$$

(1)

where M and D are the mutation (C vs. MA) and diet (high vs. low quality) effects respectively and Line denotes the $n = 19$ different haplotypes. Line and all interaction terms with it were modelled as random effects while M, D and M × D were fixed effects. Significance of the random effects was determined via likelihood ratio tests comparing models that included and excluded the term in question. Since the unit of replication in this analysis is the hemiclone line, statistical significance of treatment effects reflects both their magnitude and their degree of consistency across the 19 haplotypes.

We tested the prediction of alignment of the effects of the mutation and diet treatments by correlation analysis at the among-trait level. Alignment should be reflected in a positive correlation among traits of the mutation and diet effect coefficients, and Pearson correlation analysis is appropriate because all analyses were carried out on standardised trait values such that coefficients are comparable among traits. Note that alignment cannot be reliably confirmed or rejected for a single trait. If effects of mutation and diet treatments were in opposite directions for a particular trait, this would suggest non-alignment (or anti-alignment), but such a pattern could come about by chance if the effects of one or both treatments were weak. Likewise, if effects of mutation and diet were in the same direction for a particular trait, this would suggest some degree of alignment, but such a pattern could also come about by chance. However, although the magnitude of the mutation and diet effects depends on the particular manipulations used (i.e. the number of generations of mutation accumulation and the nutrient concentrations in the experimental diets), their effects should be correlated across traits if these effects are mediated in each case through their impact on condition. The degree of alignment can, therefore, be quantified as the strength of the positive correlation, among traits, between the effect coefficients for mutation and diet. Deviations of particular traits from the overall trend can also reveal interesting exceptions (such as the sexcombs: see Results). Analyses were performed in JMP version 11.2.1, Statistica version 7.1 (StatSoft Inc., Tulsa, OK, USA), and SAS version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Mean effects of genetic and environmental quality

Genetic quality (mutation load) exerted a substantial and significant effect only on PC1 of the morphological trait matrix in females (i.e. female body size), and did not significantly affect any morphological PCs in males (although effect sizes exceeded 0.1 for PC1 and PC4) (Table 1; Fig. 2a and b, S4). In analyses of separate morphological traits, genetic quality had significant effects on all female traits except head length, but only one male trait (wing length) (Table 2, Fig. 3). Genetic quality effect coefficients were positive (indicating that high mutation load reduced trait size) for all morphological traits except the male sexcombs, for which a small, negative effect coefficient was obtained. For CHC principal components, we detected significant mutation load effects on PC4 in females and PC1 and PC5 in males (Table 1; Fig. 2c and d).

Environmental quality (larval diet) exerted significant effects on PC1 (body size) and PC2 of the morphological trait matrix in females, but only PC1 (body size) in males (Table 1; Fig. 2a and b, S4). In analyses of separate morphological traits, environmental quality had significant effects on all female traits, and all male traits except head length (Table 2; Fig. 3). There were no significant effects on female CHC phenotype but, in males, diet significantly affected PC2, PC3 and PC6 (Table 1; Fig. 2c and d).

The mutation × diet interaction effect was moderately strong and significant for some female traits including PC1 of

the morphological trait matrix (i.e. body size), and four of the six morphological traits. In all of these cases, the effect of mutation load was stronger in females reared on a high-quality larval diet (Table 1, 2; Fig. S4). In contrast with these morphological traits, PC5 of female CHC phenotype responded more strongly to mutation load in females reared on a low-quality larval diet. In males, mutation × diet interactions were weak and non-significant in all cases.

Alignment of genetic and environmental effects

We observed qualitative alignment between the effect coefficients (i.e. average effects across haplotypes) of mutation and diet on most morphological traits and their principal components (Fig. 2a and b, 3). High mutation load and a low-quality larval diet both resulted in reduced body size and reduced head, leg and wing dimensions in both sexes, as expected for condition-dependent traits. This pattern was reflected in strong to moderate positive correlations between effect coefficients for mutation load and diet quality treatments among morphological PCs (females: $r = 0.99$, $N = 6$, $P = 0.0002$; males: $r = 0.90$, $N = 6$, $P = 0.0139$) and separate morphological traits (females: $r = 0.86$, $N = 6$, $P = 0.0293$; males: $r = 0.69$, $N = 7$, $P = 0.0836$). Contrary to the pattern observed for other morphological traits, male sexcomb width was positively and significantly affected by larval diet quality, but entirely unaffected by mutation load (Fig. 3b).

In contrast with the morphological traits, there was little evidence of alignment of genetic and environmental effects among principal components of CHC phenotype in either sex. Mutation and diet effects were opposite in direction for two of six PCs in females and three of six PCs in males (Fig. 2c and d). Lack of alignment for the CHC traits was reflected in weak and non-significant correlations among traits of the effect coefficients for mutation load and larval diet (females: $r = 0.44$, $N = 6$, $P = 0.38$; males: $r = 0.35$; $N = 6$, $P = 0.50$).

Variation among haplotypes

Among morphological trait PCs, line (haplotype) effects accounted for a substantial proportion of the variance for PC1 (body size) and PC2 in females, and for PC2 and PC4 in males (Table 1). In analyses of separate morphological traits, line effects were substantial for head length and fore-tibia length in females and head length and sexcomb width in males (Table 2). Sizeable line effects were observed for most CHC principal components in both sexes (Table 1). The mutation × line interaction was also important for most morphological and several CHC traits (Tables 1 and 2), exceeding 20% of total variance for male and female morphological PC1 (body size), female PC3 and male PC6, as well as PC2 of male CHC phenotype. The strength of the mutation × line interaction contrasted sharply with that of the diet × line interaction, which accounted for a substantially smaller proportion of variance for every trait and PC (Fig. 4). The mutation × diet × line interaction was relatively unimportant for most traits, accounting for > 10% of the residual variance only for PC3 of female CHC phenotype and for male wing length.

Table 1 Mixed model results from the separate analyses of principal component scores for morphological traits and CHC phenotype[†] in females and males. Model coefficients are reported for the fixed effects of mutation (M), diet (D) and their interaction[‡], and (unbounded) variance components for the random effects of hemiclonal line (L) and its interactions with mutation and diet. Positive coefficients for the mutation and diet effects represent larger trait values in control relative to mutation accumulation, and high-quality relative to low-quality larval diets respectively

	Females							Males						
	Fixed effects			Random effects				Fixed effects			Random effects			
Traits	M	D	M × D	L	M × L	D × L	M × D × L	M	D	M × D	L	M × L	D × L	M × D × L
Morphological trait principal components														
PC1	**24.60***	**26.92****	**9.69***	9.0	**21.8****	1.8	0.8	10.72	**21.59****	−0.67	−0.3	**25.7***	2.4	5.9
PC2	−3.20	**−6.90***	−2.09	**15.2***	**6.9***	1.7	**−5.6**	**17.4***	−5.29	0.24	17.4	6.8	−1.0	4.6
PC3	5.96	6.67	1.59	2.5	**20.8****	3.3	−2.2	−6.53	2.59	−1.91	8.0	**12.5***	1.4	−1.6
PC4	3.19	−0.89	5.19	−0.5	6.5	−1.5	1.8	−11.51	−2.81	−4.56	15.6	**16.1***	−2.8	4.2
PC5	−1.04	−6.18	4.97	1.5	**12.3**	0.9	4.4	−6.52	−7.38	2.31	1.5	**8.4**	1.4	−3.4
PC6	2.59	2.82	−2.56	2.1	**11.5***	1.7	−1.9	−8.69	−6.05	6.42	−3.3	**22.8***	−0.9	0.6
Cuticular hydrocarbon trait principal components														
PC1	0.19	0.82	2.06	**55.3****	5.5	3.1	4.7	**−13.39**	5.70	0.71	7.9	**6.7**	4.4	−0.3
PC2	3.73	8.78	1.26	**10.2**	5.3	1.0	1.6	18.61	**10.81****	2.06	−6.7	**42.5****	0.7	1.3
PC3	1.85	−7.79	−4.55	1.4	**12.7**	1.6	**12.1****	−7.21	**4.57**	2.32	**9.3***	**4.8***	−0.2	0.8
PC4	**9.33**	4.01	−4.00	**15.7****	**4.9**	−0.6	1.4	7.07	2.35	1.53	4.2	3.4	−0.6	1.3
PC5	0.58	−0.35	**4.86**	**3.4**	1.7	0.3	0.6	**−5.06**	−1.02	−0.09	**4.7****	1.0	0.2	0.4
PC6	−1.23	−0.90	1.15	2.2	2.1	0.0	**2.2****	2.15	**−3.20**	0.41	1.9	**1.7***	0.3	−0.5

Bold font denotes $P < 0.05$; *$P < 0.01$; **$P < 0.001$.
[†]PCs represent different combinations of morphological or CHC traits in females and males. Loadings for morphological and CHC traits are given in Tables S2 and S3, and Tables S4 and S5 respectively.
[‡]For display purposes, fixed and random effect coefficients have been multiplied by 100.

Table 2 Mixed model results from the separate analyses of standardised morphological traits[†] in females and males. Model coefficients are reported for the fixed effects of mutation (M), diet (D) and their interaction, and (unbounded) variance components for the random effects of hemiclonal line (L) and its interactions with mutation and diet[‡]. Positive coefficients for the mutation and diet effects represent larger trait values in control relative to mutation accumulation, and high-quality relative to low-quality larval diets respectively.

	Females							Males						
	Fixed effects			Random effects				Fixed effects			Random effects			
Traits	M	D	M × D	L	M × L	D × L	M × D × L	M	D	M × D	L	M × L	D × L	M × D × L
Thorax length (TL)	**21.79***	**21.46****	**11.52***	8.0	**17.0****	1.3	−0.1	10.64	**17.07****	3.02	1.1	**24.6***	−3.2	**9.5***
Head length (HL)	10.00	**8.41***	3.32	**15.8***	**9.6****	**3.3**	**−5.2**	0.15	2.63	1.66	**11.8**	6.7	−1.0	4.4
Head height (HH)	20.14	**19.55****	**8.99***	7.8	**15.1***	1.8	1.1	7.04	**14.50***	−3.27	1.5	**15.5****	3.4	−1.7
Head width (HW)	22.37	**19.79****	**10.52***	5.7	**24.0****	2.8	2.8	10.13	**16.81***	−1.49	0.0	**17.8***	4.5	2.1
Fore-tibia length (FL)	**14.52**	**19.97****	5.23	**10.1**	6.6	1.2	1.3	6.16	**13.34***	0.35	3.2	**18.1****	1.8	0.6
Wing length (WL)	**23.24**	**25.98****	6.69	2.6	**25.0****	1.6	−2.3	20.31	**18.93****	−1.15	5.4	**22.7***	−4.3	**11.5****
Sex comb width (CW)	–	–	–	–	–	–	–	−0.10	**15.04***	−2.82	14.0	**10.7***	2.2	0.2

Bold font denotes $P < 0.05$; *$P < 0.01$; **$P < 0.001$.
[†]Traits values were converted to z-scores (mean = 0, variance = 1) prior to analysis.
[‡]For display purposes, fixed and random effect coefficients have been multiplied by 100.

DISCUSSION

Our results furnish partial support for widely held assumptions from condition-dependence theory, but also reveal striking and unexpected differences between the effects of genetic and environmental quality on the expression of morphological and CHC traits in *D. melanogaster*. These findings challenge established conceptual models about how genetic and environmental variation influences the expression of condition-dependent traits, and the potential for such traits to signal 'good genes'.

The prediction of alignment of the effects of genetic and environmental quality on trait expression was supported for some traits. As expected, two fitness-related traits (female body size and male wing length) exhibited consistent and significant responses among haplotypes (hemiclonal lines) to both mutation and diet treatments, showing enhanced expression under both low mutation load and high diet quality. Moreover, most morphological traits and their principal components responded in the same direction to both genetic and environmental quality manipulation (Figs 2a and b, 3). However, one fitness-related morphological trait (the male
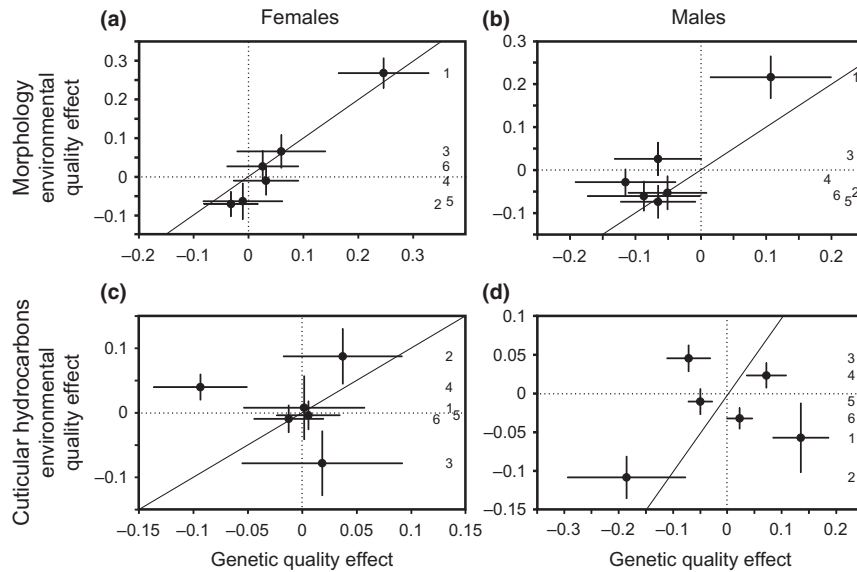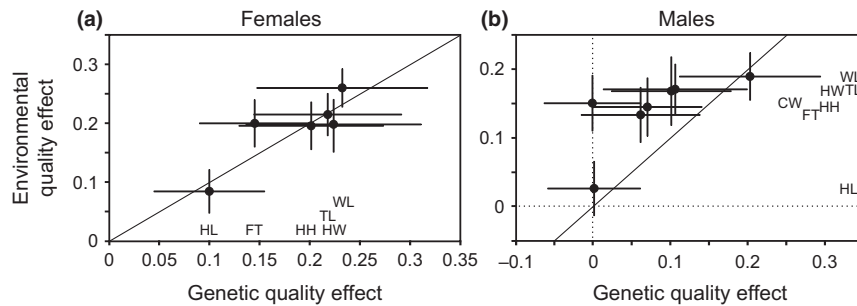
Figure 2 Effects of genetic quality (mutation load) and environmental quality (larval diet nutrient concentration) on principal components (PCs) of the morphological trait matrix in (a) females and (b) males, and PCs of the CLR-transformed CHC matrix in (c) females and (d) males. In analyses of PCs, effect direction is arbitrary but mutation and diet effects are still expected to align (i.e. be positively correlated). Effect coefficients (and associated SEs) from mixed models (Table 1) are shown. Trait labels correspond to PCs 1–6. The solid diagonal is a line of equality (x = y).

Figure 3 Effects of genetic quality (mutation load) and environmental quality (larval diet nutrient concentration) on separate morphological traits (standardised values) in (a) females and (b) males. Positive effects indicate trait enlargement under low mutation load or high larval diet quality. Effect coefficients (and associated SEs) from mixed models (Table 2) are shown. Trait labels correspond to Table 2. The solid diagonal is a line of equality (x = y).

sexcombs) responded strongly and consistently to one treatment but showed no overall response to the other treatment. Likewise, we found little evidence of alignment of effects of genetic and environmental quality on CHC phenotype (Fig. 2c and d).

Lack of alignment for these traits reflected, in part, a striking difference between the effects of genetic and environmental quality in terms of their consistency among haplotypes. The response of most traits to diet manipulation was consistent across lines (as reflected in small diet × line variance components: Figs 4, S6), similar to the results of a previous study which found no evidence of variation in response to a larval density manipulation among a different set of *D. melanogaster* hemiclones (Rode & Morrow 2009). In contrast, mutation effects on most traits varied among lines in both magnitude and direction (as reflected in relatively large mutation × line variance components: Figs 4, S6). Such extensive variation among haplotypes in the effect of mutation load

weakened the overall effect of this treatment for some traits. Interestingly, while haplotype-specific responses were predicted for traits weakly associated with fitness (such as female traits other than body size), such responses were not expected for traits with close links to fitness, for which genetic quality effects are assumed to be mediated by condition and are therefore expected to be consistent across haplotypes. The male sexcomb starkly illustrates this pattern. The sexcomb is a male-limited secondary sexual trait (Ahuja & Singh 2008; Ng & Kopp 2008; Ahuja *et al.* 2011), although it is unclear to what extent this structure has been sexually selected for size exaggeration or how costly it is to express. Simply finding weak condition dependence of sexcomb width would not therefore have been very surprising (e.g. see Johnstone *et al.* 2009). In contrast, we found that the sexcomb responded strongly and consistently to environmental quality, but genetic quality effects were highly haplotype-specific, and near zero on average, both in analyses with and without thorax length
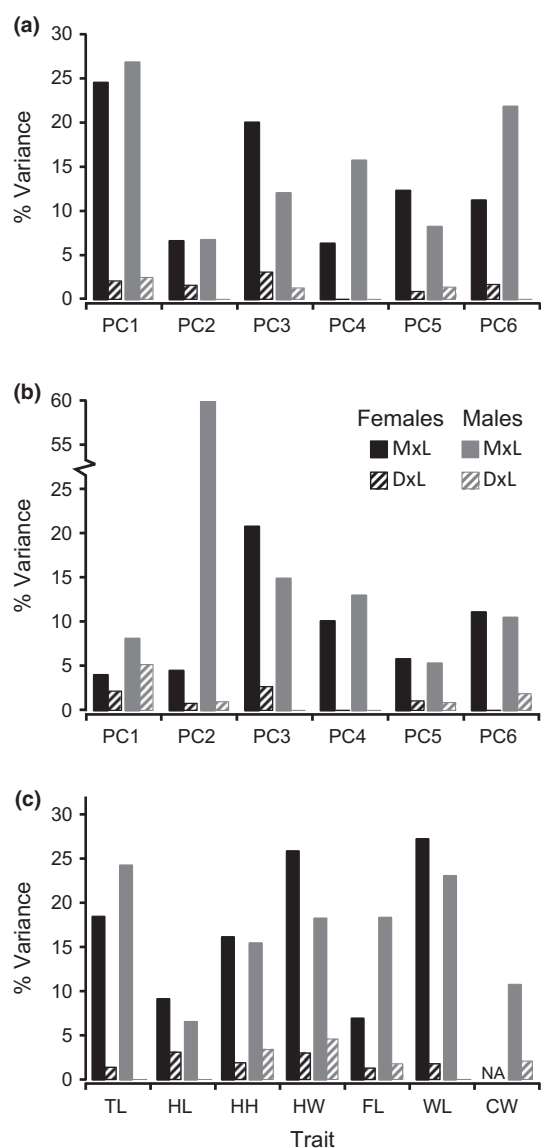
Figure 4 Variance components in males and females for the mutation × line (MxL) and diet x line (DxL) interactions for principal components of the morphological trait matrix (a), principal components of the CLR-transformed CHC matrix (b) and separate morphological traits (c). Note that principal components are based on different trait combinations in each sex. Variance components were estimated in linear mixed-effects models (Tables 1 and 2). For each trait, the percent of total variance accounted-for by each variance component is plotted, with negative variance components set to zero.

included as a covariate (Table 2, S6, Fig. 3, S5). The sexcomb is therefore a condition-dependent secondary sexual trait that fails to signal genetic quality.

The prediction of stronger responses by traits that are more closely associated with fitness was weakly supported. As expected, the magnitudes of response to both genetic and environmental quality were large for female body size (which is under strong fecundity selection), relative to other morphological traits. Likewise, the responses of principal components for male CHCs (which are sexually selected via their role as sexual signals) were generally larger than those of principal

components for female CHCs (which do not appear to be subject to strong sexual selection). However, the responses of other fitness-related traits were not strong in comparison with non-sexual traits. For example, the responses of the male sexcombs and wings, which play a role in sexual signalling, were comparable in magnitude to those of female wings, fore-tibia and head height and width, which are not known to have strong links to fitness, and such a pattern was observed even when examining responses in these traits relative to thorax length (Table S6; Fig. S5), suggesting that this pattern was not driven entirely by body size variation. Indeed, the strong responses of female (but not male) morphological traits (e.g. female head height, head width, fore-tibia length), detectable in analyses of both absolute (Table 2) and relative trait sizes (Table S6), is surprising, as such female traits are likely under weak stabilizing selection. It is possible that the developmental sensitivity of such metric traits to environmental quality arises as a pleiotropic effect of alleles that confer condition-dependent expression of other traits with closer links to fitness.

The patterns outlined above suggest the existence of two distinct forms of condition dependence. One form links trait expression to both genetic and environmental quality, as predicted by theory (Andersson 1982; Nur & Hasson 1984; Rowe & Houle 1996; Hill 2011; Emlen et al. 2012). For traits exhibiting this form of condition dependence, the effects of both genetic and environmental quality appear to be mediated primarily through condition, and are therefore aligned in their effects (Fig. 1a). Such traits can function as indicators of 'good genes', while also responding strongly to variation in environmental quality. Another form of condition dependence links trait expression primarily or exclusively to environmental quality. Such environmental condition dependence is consistent with a different model, whereby the expression of some traits depends on the ability to acquire specific resources (e.g. certain micro-nutrients) and thereby optimise specific aspects of cell function, rather than on overall condition (Fig. 1b). The expression of such traits may remain sensitive to environmental perturbations that alter resource levels or disrupt specific biochemical pathways. However, consistent effects of overall genetic quality (i.e. mutation load) may not be detectable if trait expression depends largely on aspects of performance affected by only one or a few loci, representing a small mutational target. Rather, for traits exhibiting this form of condition dependence, the unique mutations acquired by different genotypes (and associated epistatic interactions) may alter patterns of resource allocation among different traits, resulting in either increased or decreased trait expression under high mutation load relative to controls, instead of the consistent, negative effect predicted by theory. Such genotype-specific deviations may be especially strong if a relatively large number of loci control the pattern of resource allocation, and these loci therefore represent a substantial mutational target. In contrast with the effects of resource acquisition loci, which are assumed to be mediated through condition and therefore expected to affect trait expression in a consistent direction across genotypes, mutations in resource allocation loci may increase or decrease relative allocation to a particular trait at any level of condition.

These ideas could be tested by examining the extent to which the expression of particular traits is limited by specific resources and the functionality of specific biochemical pathways, and by elucidating the relative sizes of mutational targets represented by loci affecting resource acquisition vs. resource allocation. For example, it has been shown that the availability of particular macronutrients in the larval diet has differential effects on the expression of sexual and non-sexual morphological traits in the flies *Drosophila melanogaster* (Shingleton *et al.* 2009) and *Telostylinus angusticollis* (Sentinella *et al.* 2013). More fine-grained studies are needed to determine whether the expression of certain traits is limited by particular micro-nutrients (e.g. specific amino acids) and, crucially, whether the acquisition of particular micro-nutrients and the functionality of particular biochemical pathways are subject to locus-specific genetic effects.

The lack of a consistent and significant effect of genetic quality on the means of most of the traits examined is unlikely to have been caused by a weak genetic manipulation. Our MA treatment allowed deleterious genetic mutations (and perhaps also epimutations) to accumulate for 50 generations, resulting in substantially reduced fitness in the MA lines, including a 31% reduction in juvenile viability relative to controls (Mallet *et al.* 2012). This contrasts with our diet manipulation, which had no detectable effect on juvenile viability (Fig. S1) and was therefore arguably mild in comparison with our genetic quality manipulation, as well as with natural variation in resource quality. Another potential reason for failing to detect effects of genetic quality is that benign laboratory conditions could eliminate many of the challenges involved in foraging in natural environments. This could reduce the mutational target for resource acquisition and thereby weaken the signal of mutation load in trait expression. However, our results provide little support for this interpretation. If the effect of high mutation load was strongly environment-dependent, we would expect the effects of high mutation load on trait means to be exaggerated when resources are limited, but we found little evidence of such a pattern. Thus, the lack of a consistent signal of genetic quality on most traits in this study cannot be explained as a laboratory artefact.

Although male traits that fail to reveal genetic quality fall outside the scope of 'good genes' models of sexual coevolution, signals of environmental quality may reveal direct benefits such as fecundity or fertility and could therefore drive the evolution of costly mating preferences (e.g., Wolf *et al.* 1997). Environmentally sensitive traits could also serve as honest signals of offspring quality, even in species lacking obvious forms of paternal investment: in several species, including *D. melanogaster*, it has been shown that components of environmental variation are transmitted to offspring via non-genetic paternal effects, and theory suggests that such paternal effects can support the evolution of costly female preferences (Bonduriansky & Day 2013).

While existing theory has provided important insight into the evolution of condition dependence for costly signalling traits, this body of theory is clearly insufficient to provide a full description of the complex development of all traits. Empirical studies have shown that fitness components often

(Cotton *et al.* 2004), but not always (Bolund *et al.* 2010), exhibit heightened condition dependence. There is also abundant genetic variation in many (Wilkinson & Taper 1999; David *et al.* 2000; Kotiaho *et al.* 2001; Forstmeier *et al.* 2012), but not all (Walsh & Blows 2009), condition-dependent traits, and sexual ornaments often, but again not always, exhibit greater inbreeding depression compared to other traits (Prokop *et al.* 2010). Crucially, the key assumption that the expression of condition-dependent traits reflects genetic variation across many loci involved in resource acquisition and processing efficiency (Rowe & Houle 1996) has not received consistent support (Tomkins *et al.* 2004; Schielzeth *et al.* 2012). Our findings may help to reconcile these seemingly conflicting results. We show that different phenotypic traits appear to exhibit different forms of condition dependence, with some traits responding consistently to both genetic and environmental quality but other traits responding predominantly or purely to environment. Such environmental condition dependence could occur in traits for which expression is affected by allelic variation in resource allocation genes, but is not strongly dependent on genetic quality throughout the genome. The possibility of an environmental form of condition dependence has important implications for empirical studies that have traditionally used a trait's response to environmental manipulation as a proxy for the trait's potential to signal genetic quality. Further studies are needed to illuminate the developmental mechanisms involved in these distinct forms of condition dependence, map their distribution among trait types (e.g. sexually selected vs. fecundity-selected, male vs. female, morphological vs. life history), and reveal the regimes of selection that drive their evolution.

AUTHORSHIP

RB, MAM and HDR conceived the study and MAM derived the hemiclonal lines and performed mutation accumulation on them. Cuticular hydrocarbon samples were collected by MAM, DA and HDR, subsequent gas chromatography and data integration were performed by DA, and VPG and JJE handled the compositional data transformation. RB collected the morphological data. Statistical analyses were performed by RB and HDR. RB and HDR wrote the manuscript with input from all the authors.

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