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Antagonistic pleiotropy for life-history traits at the gene expression level

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Life-history trade-offs prevent different components of fitness from being maximized simultaneously. Although the existence of trade-offs has been clearly demonstrated, the 'classical' mechanism of adaptive resource allocation that should underlie them has recently received criticism. In this study, we explore the molecular mechanisms of life-history trade-offs by applying a quantitative genomic approach. Analysis of global gene expression in *Drosophila melanogaster* revealed 34 genes whose expression coincided with the genetic trade-off between larval survival and adult size. The joint expression of these candidate 'trade-off' genes explained 86.3% of the trade-off. Fourteen of these genes have known functions which suggest that the larval survival–adult size trade-off could be the result of resource allocation at the organismal level, but at the level of cellular metabolism the trade-off would reduce to a shift between energy metabolism versus protein biosynthesis, regulated by the RAS signalling pathway.

Keywords: life-history evolution; genetic trade-off; antagonistic pleiotropy; quantitative genomics; global gene expression

1. INTRODUCTION

Life-history traits are often genetically negatively correlated (Barton & Turelli 1989) as the result of simultaneous selection on two correlated traits. A positive genetic correlation between life-history traits will soon lead to fixation of any particular allele. Only negative correlations between life-history traits persist (Rose 1983). The loci involved in life-history trade-offs are postulated to show antagonistic pleiotropy: each locus influences both traits, but does so in opposite direction (Stearns 1992). Consequently, fitness will be maximized but a further increase in one trait (i.e. fecundity) will decrease the other (i.e. longevity). The physiological mechanism underlying this pattern should involve competition for some common limiting substance. In other words, life-history trade-offs are the result of adaptive resource allocation of limiting resources (Zera & Harshman 2001).

This explanation for life-history trade-offs has empirical support (Zera & Harshman 2001), but no specific molecular mechanisms have been put forward. No actual loci are known that exhibit antagonistic pleiotropy on two correlated life-history traits. By contrast, developmental genetic studies in *Caenorhabditis elegans* have suggested that trade-offs could be the result of variation in molecular signalling, and not caused by resource allocation (Leroi 2001).

Although the 'resource allocation' hypothesis and the 'molecular signalling' hypothesis of life-history trade-offs are not necessarily mutually exclusive, the issue is of sufficient importance to be pursued in more detail.

Given such potentially divergent approaches to life-history trade-offs, the problem that needs attention is: does negative pleiotropy in genes affecting life-history traits exist? If so, are these genes involved in resource allocation or rather in signal transduction?

The way to address this issue is to search for genes that affect in opposite ways two life-history traits that are known to be negatively correlated. Adaptive variation in quantitative traits might be the result of variation in gene expression rather than allelic differences (Purugganan 2000; Barrier *et al.* 2001; Mackay 2001; Bochdanovits *et al.* 2003). Consequently, in this study we explored global gene expression in staged third instar larvae of nine isofemale lines of *Drosophila melanogaster*. For the same strains, two life-history traits—pre-adult survival and adult body weight—have been measured. These two traits were expected to exhibit a genetic trade-off. If quantitative trait variation is the result of variation in transcript levels rather than of allelic differences, and if the genes involved exhibit antagonistic pleiotropy, we may expect to find genes whose expression correlates positively with one trait but negatively with the other. Such genes would qualify as 'trade-off' genes. The question then remains whether they are involved in resource processing or in molecular signalling.

2. MATERIAL AND METHODS

(a) *Fly stocks and experimental conditions*

Five isofemale lines from Wenatchee (WA, USA) and four isofemale lines from San José (Costa Rica) were collected in the summer of 2001 and kept on standard corn medium at 17.5 °C until the start of the experiment. Prior to the experiment, the lines were reared at the experimental temperature for one generation to rule out possible effects of parental rearing temperature. Larvae were raised at low density under unlimited food conditions. The experiment was conducted at 27.5 °C on standard corn medium stained with 0.05% bromophenol blue. This medium has no effect on larval growth and allows for accurate staging of third instar larvae just prior to pupariation at their maximum size (Andres & Thummel 1994). Adult males that emerged from the vials from which the larvae were previously collected were weighed in groups of five to the nearest 0.01 mg on a Sartorius microbalance (Bochdanovits *et al.* 2003). In a separate experiment, larvae from the same isofemale lines were raised at low density (exactly 50 eggs per jar) and under unlimited food conditions. From these vials all larvae were collected at the same time as the staged larvae used for the gene expression analysis. The number of larvae collected added to the number of the occasional pupae already present in the vials was taken as a measure of pre-adult survival.

(b) *Gene expression analysis*

Using a paintbrush, larvae with dark blue guts were manually collected from the surface of the medium and were immediately frozen in liquid nitrogen. Approximately 20 larvae were used for isolating mRNA with a Qiagen Direct mRNA kit. From *ca.* 3 μg of mRNA, Bio-11-CTP- and Bio-11-UTP-labelled aRNA were prepared using standard Affymetrix protocols (http://www.affymetrix.com/Download/manuals/expression_ever_manual.pdf). Hybridization and scanning were performed on an Affymetrix Fluidics Station 400 and a GeneArray Scanner at the Leiden Genome Technology Centre. The raw data were normalized using MICROARRAY SUITE, v. 5.0 prior to further analysis.

(c) *Data analysis*

To allow for meaningful statistical analysis we selected probes that had been detected on at least eight out of the nine microarrays. This filtering resulted in 1670 probes for further analysis. For each gene, two correlation coefficients were calculated: one was between the

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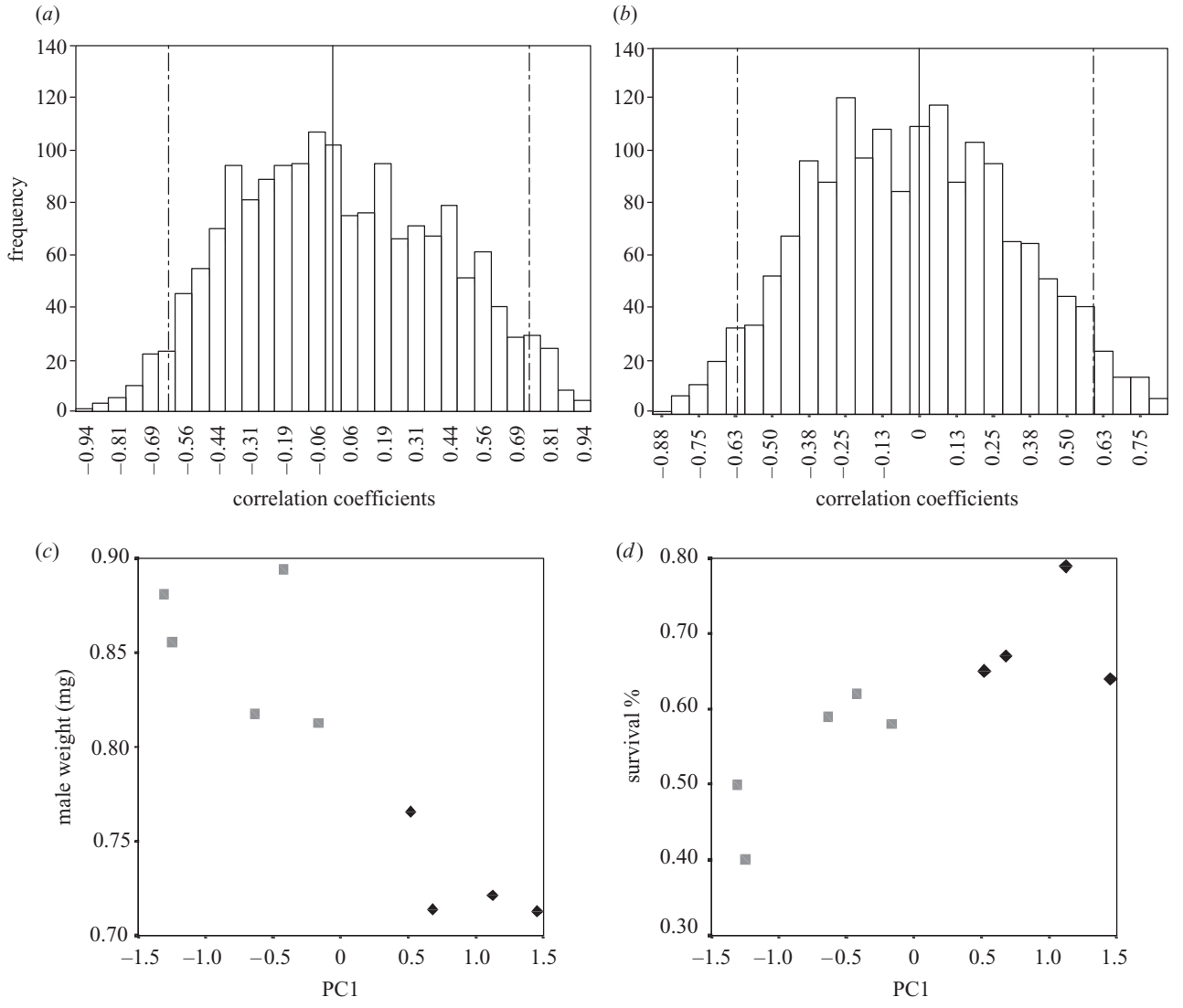

Figure 1. (*a*) and (*b*) represent the frequency distribution of the correlation coefficients between (*a*) gene expression of 1670 genes and male weight and (*b*) gene expression of 1670 genes and larval survival. The dashed lines indicate the cut-off values for the 3.5% most extreme correlation coefficients, which result in two expected FPs for a dataset of 1670 values. The cut-off values for the correlation coefficients between gene expression and male weight were −0.62 and 0.74, respectively, and for the correlation coefficients between gene expression and pre-adult survival were −0.62 and 0.59. (*c*) and (*d*) represent the scatter plots between PC1 derived from the gene expression data on the 34 candidate 'trade-off' genes and male weight (*c*) and larval survival (*d*). Squares, isofemale lines from Wenatchee, WA; diamonds, isofemale lines from Costa Rica.

measure of gene expression of that gene and body size, and the other was between the measure of gene expression of this gene and pre-adult survival. All calculations were performed on isofemale line means. Genes that showed antagonistic pleiotropy were defined as those genes that occupied the opposite tails of the two frequency distributions of correlation coefficients, i.e. exhibited strong negative correlation with one trait but strong positive correlation with the other trait. Owing to chance alone, there will be genes that are present in the two extremes at the same time. These genes are false positives (FPs) and their number depends on how wide the 'lower' and 'upper' tails are set. The cut-off values could be set arbitrarily, but for each cut-off value a certain number of expected FPs will be present in the set of candidate genes (CG). The expected probability of one gene being present in the opposite tails of the two distributions simultaneously just by chance alone is the product of the observed frequencies of coefficients that are greater than or equal to the cut-off value. This expected probability multiplied by the number of genes in the dataset (1670) gives the expected number of FPs. For a series of cut-off values, the number of expected FPs and the number of CGs were determined. Lower FPs coincided with a lower ratio of FP/CG (i.e. higher sensitivity), but not linearly. At FPs of less than 4, FP/CG decreased only slightly with a further decrease of FP, from 0.15 (FP = 4.2) to 0.1 (FP = 1). For this analysis we chose to accept FP = 2, FP/CG = 0.12. A further decrease in FP did not have a substantial effect on FP/CG, but would decrease the number of CGs from 34 to 20. This would probably result in information loss. Given 1670 probes, this procedure results in considering genes that occupy the lower and upper 3.5% of the distributions ($0.035 \times 0.035 \times 1670 = 2.04$) (see figure 1 legend). A principal component (PC) analysis on the gene expression data was performed in SPSS, v. 10.0. A two-way analysis of covariance (SPSS, v. 10.0) was used, with PC1 as a dependent variable and pre-adult survival and male weight as covariates, to measure the percentage of explained variance in the data.

3. RESULTS

(a) *Detection of genes with antagonistic pleiotropy*

A negative correlation was observed between the family means of pre-adult survival and male weight ($r = -0.683$, $p = 0.04$, $n = 9$), showing the expected trade-off. The correlation coefficients between gene expression and both

Table 1. Thirty-four candidate 'trade-off' genes from the categories 'low weight, high survival' and 'high weight, low survival'. A description of the genes can be found in the flybase database (http://flybase.bio.indiana.edu); annotation of molecular and biological functions were derived from the gene ontology (http://www.ebi.ac.uk/ego) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.org) databases.

	name	molecular function	biological function
low weight, high survival	CG3244		
	Lcp1	larval cuticle protein	contributes to the structural integrity of the cuticle of an insect larva
	CG15308		
	CG17738		
	CAH1	carbonic anhydrase 1	energy metabolism
	CG7567		
	CG10622	citrate cycle; succinate-CoA ligase	carbohydrate metabolism
	CG14454		
	CG10311		
	CG15353		
	CG11300		
	CG2233		
	CG7834	electron transport flavoprotein, works in conjunction with acyl-CoA dehydrogenase	mitochondrial electron transport system
	CG8661		
	CG8145	nucleic acid binding	
high weight, low survival	*Smt3*	protein tagging; ubiquitin-like protein	cell growth and maintenance
	CG1249		
	CG14957	mRNA splicing	cell growth and maintenance
	BcDNA:GH02250		
	CG12581		
	CG18239		
	Arf51F	ADP ribosylation factor 51F	cell growth and maintenance
	CG12868		
	CG3164	ATP-binding cassette (ABC) transporter	nucleotide binding and transport
	Est-P	carboxylesterase	
	CG14483		
	BcDNA:GH09045		RAS protein signal transduction
	CG5739		
	CG15219		
	CG3843	ribosomal protein L10A; structural constituent of ribosome	protein biosynthesis
	CG18449		
	ImpE2	ecdyson inducible gene E2	imaginal disc eversion–imaginal disc morphogenesis
	CG5171	trehalose phosphatase	
	CG18078	RNA-binding	

male weight and pre-adult survival were calculated (figure 1*a*,*b*). Genes that showed antagonistic pleiotropy were those that occupied the lower tail of one distribution and the upper tail of the other distribution simultaneously. The cut-off values for the tails were set to 3.5% for two expected FPs (see § 2). If the observed number of genes present in both the lower 3.5% of one distribution and the upper 3.5% of the other was considerably higher than two, those genes would qualify as 'trade-off' genes. Using this approach, we detected 15 'low weight, high survival' genes and 19 'high weight, low survival' genes (table 1). The probability of getting 34 (or more) hits when four are expected can be calculated from the Poisson distribution and equals 1.08×10^{-10}. Hence, it was extremely unlikely that this set of candidate genes was detected by chance.

(b) *Quantifying antagonistic pleiotropy at the gene expression level*

These 34 genes exhibited antagonistic pleiotropy in gene expression on two life-history traits in isofemale lines from natural populations. To quantify the joint effect of the expression of these 34 candidate genes on the two life-history traits, we extracted the information contained in the data in one derived variable. This derived variable, the first principal component (PC1), contained over 70% of the variation present in the expression of the 34 genes and was subjected to correlation tests with both pre-adult survival and male weight. This combined measure of gene expression showed significant and very high correlation with pre-adult survival ($r = 0.830$, $p = 0.006$, $n = 9$) and male weight ($r = -0.906$, $p = 0.001$, $n = 9$) (figure 1*c*,*d*).

PC1 explained 86.3% of the observed trade-off, measured in a two-way analysis of covariance. Tellingly, PC1 correlated better with both life-history traits than they did with each other.

4. DISCUSSION

The first question asked in this study was whether genetic trade-offs are the result of genes exhibiting antagonistic pleiotropy on life-history traits. We detected 34 genes with expression exhibiting antagonistic pleiotropy. These genes explained most of the genetic trade-off between pre-adult survival and male weight. The next issue is to elaborate on the probable mechanism. Population genetics and physiological studies indicated that genetic trade-offs are the result of resource allocation (Zera & Harshman 2001). However, valid observations have suggested that trade-offs could be induced by molecular signalling (Leroi 2001).

Available data on the annotation of the candidate 'trade-off' genes (gene ontology, see http://www.ebi.ac.uk/ego; KEGG, http://www.kegg.org) are summarized in table 1. Four out of 15 (26.7%) 'low weight, high survival' genes and 10 out of 19 (52.6%) 'high weight, low survival' genes have known molecular and/or biological function.

All four annotated 'low weight, high survival' genes seemed to be causally involved with the observed trade-off. Larval cutical protein 1 is a structural component of the larval cuticle. High expression of this protein might be an investment in larval defence. Genotypes that did so produced higher pre-adult survival at the cost of adult weight, in agreement with the predictions of adaptive resource allocation. The three other genes were all enzymes involved in energy metabolism. Genotypes with increased energy metabolism were predisposed to have a higher larval survival at the cost of adult size. This finding also supported the resource allocation hypothesis.

Three 'high weight, low survival' genes had previously known molecular functions but no obvious involvement with any biological processes. An ecdysone-inducible gene (*ImpE2*) is probably not related to the trade-off, but confirmed that the intended developmental stage, i.e. just prior to pupation, has been used. The other six genes had biological functions that seemed relevant for determining adult weight. Two genes had functions in protein metabolism, a third was involved in mRNA splicing and a fourth was a structural constituent of ribosomes (L10A). The first three were categorized as 'cell growth and/or maintenance' genes and all are involved in protein biosynthesis. In addition, one nucleotide transporter gene was detected that is plausibly related to DNA synthesis, thus cell division and/or growth. These results suggested that genotypes with enhanced larval protein biosynthesis and/or cellular growth were predisposed to grow large at the cost of their larval survival, in line with the hypothesis of adaptive resource allocation.

However, the last annotated 'high weight, low survival' gene adds an interesting complexity to the issue. BcDNA:GH09045 is part of the RAS signalling pathway; RAS is known to be associated with growth regulation (Ayllon & Rebollo 2000). In our data, naturally occurring genotypes with higher RAS signalling activity were larger, at the cost of larval survival. Our interpretation is the following. Adaptive resource allocation does underlie the larval survival versus adult size trade-off, and is caused by differences is cellular metabolism, i.e. protein biosynthesis (growth) versus energy metabolism (survival). However, this shift might be regulated by the RAS signalling pathway. In this view, BcDNA:GH09045 would not be a 'death signal' as proposed in *C. elegans* (Leroi 2001) but would simply shift the focus of cellular metabolism. It has been suggested before that molecular signalling could coordinate cellular metabolism (Britton *et al.* 2002), and the data presented here suggest that life-history trade-offs could be the result of adaptive resource allocation at the organismal level, mediated by signal transduction pathways at the level of cellular metabolism. Thus, the 'resource allocation' and 'molecular signalling' approaches to life-history trade-offs could easily turn out to be complementary rather than mutually exclusive, and further studies on the molecular mechanisms of antagonistic gene action should provide the answers.

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