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Temperature-driven gene expression evolution in natural and laboratory populations highlights the crucial role of correlated fitness effects for polygenic adaptation

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Abstract

The influence of pleiotropy on adaptive responses is a highly controversial topic, with limited empirical evidence available. Recognizing the pivotal role of the correlation of fitness effects, we designed an experiment to compare the adaptive gene expression evolution of natural and experimental populations. To test this, we studied the evolution of gene expression in response to temperature in two *Drosophila* species on a natural temperature cline in North America and replicated populations evolving in hot- and cold-temperature regimes. If fitness effects of affected traits are independent, pleiotropy is expected to constrain the adaptive response in both settings, laboratory and natural populations. However, when fitness effects are more correlated in natural populations, adaptation in the wild will be facilitated by pleiotropy. Remarkably, we find evidence for both predicted effects. In both settings, genes with strong pleiotropic effects contribute less to adaptation, indicating that the majority of fitness effects are not correlated. In addition, we discovered that genes involved in adaptation exhibited more pleiotropic effects in natural populations. We propose that this pattern can be explained by a stronger correlation of fitness effects in nature. More insights into the dual role of pleiotropy will be crucial for the understanding of polygenic adaptation.

Keywords: pleiotropy, gene expression evolution, correlated fitness effects, temperature adaptation

Introduction

Pleiotropy describes the phenomenon that a single gene affects multiple traits. Pleiotropic effects are well documented for many species by the observation that single mutations result in changes of multiple traits. The importance of pleiotropy for evolution has already been recognized by Fisher (1930) and later modified by Orr (1998, 2000).

Nevertheless, the extent of pleiotropy, the number of traits affected by a single mutation, is not uniform across genes. Genome-wide studies suggested that only a very small number of loci are highly pleiotropic (Wagner et al., 2008; Wang et al., 2010). Although these studies were challenged for some technical reasons (Hermisson & McGregor, 2008; Hill & Zhang, 2012a, 2012b), the observation that pleiotropic genes are more conserved (Fraser et al., 2002; Hahn & Kern, 2005; Mahler et al., 2013; Papakostas et al., 2014; Promislow, 2004; Rausher & Chang, 1999) supports the idea of different degrees of pleiotropy among genes. With a uniform mutation rate, stronger purifying selection operating on genes with a more central position in regulatory networks (i.e., a higher connectivity) (Erwin & Davidson, 2009; He & Zhang, 2006; Wagner et al., 2007) leads to the observed differences in sequence conservation. By now, the heterogeneity in the level of pleiotropy among genes is widely accepted, but the influence of pleiotropy on adaptive responses is far less understood.

The prediction is that highly pleiotropic genes are less likely to respond to selection than less pleiotropic ones because the fitness cost increases with every nonfocal trait that is affected by a given gene (cost of complexity [Orr, 2000]). The empirical evidence for this simple model is quite mixed. Gene expression in fish adapted to different temperature regimes showed that less pleiotropic genes are not only more differentially expressed between habitats but also more plastic (Papakostas et al., 2014). Nevertheless, in sticklebacks, this pattern was not observed (Rennison & Peichel, 2022). The elevated pleiotropy observed in genomic regions exhibiting parallel divergence among distinct pairs of stickleback ecomorphs indicates that pleiotropy confers a selective advantage, thereby fostering a greater degree of parallel evolution.

As pointed out by several theoretical studies (Blows, 2007; Guillaume, 2011; Lande, 1979; Lande & Arnold, 1983), Fisher's model may be too simplistic, and the outcome depends on the correlation of traits and the associated multidimensional fitness function, which translates trait values into fitness. To understand the impact of pleiotropy on adaptation, key parameters such as effect size, correlation among pleiotropic traits, and fitness function should be known. Because estimates for these key parameters are typically not available, new approaches are required to understand the role of pleiotropy for adaptation.

Here, we present a novel empirical approach aimed at investigating the role of pleiotropy in the process of adaptation.

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Our study utilizes two distinct empirical systems, both involving selection on the same focal trait. In the first system, the correlation of the fitness effect of the focal trait with the fitness effects of other traits is minimized. The second system however provides the opportunity for correlation of fitness effects. We propose that this experimental setup holds the potential to reveal synergistic pleiotropy, whereby traits affected by the adaptive response of the focal trait also contribute to adaptation to the correlated environmental variables. To test this hypothesis, we conducted a study on temperature adaptation in polymorphic *Drosophila* populations. In our first data set, we used experimental evolution to identify genes that contribute to adaptation of polymorphic *Drosophila* populations to different temperature regimes. This experimental design allowed us to limit the difference between the evolving populations to temperature, thereby minimizing the correlation of temperature with other traits. However, it is important to acknowledge that populations evolving in the two temperature regimes most likely also adapt to shared selection pressures (i.e., laboratory adaptation), but these traits are not correlated with temperature: The laboratory conditions are shared, while the temperature regime differs. Our second data set builds on natural populations collected from a temperature cline on the US East Coast (Zhao et al., 2015). Contrary to the first data set, the environment differs not only in temperature but also in many other variables, biotic and abiotic.

To assess adaptive responses, we utilized gene expression evolution as an indicator, a widely used method (e.g., El Taher et al., 2021; Hart et al., 2018; Hsu, Jakšić, et al., 2020; Li et al., 2021; Nourmohammad et al., 2017). Specifically, we identified selected genes based on significant differences in expression between populations adapted to different temperatures. The degree of pleiotropy of expressed genes can be approximated through various measures, such as the tissue specificity (*tau*), which reflects the number of tissues in which a gene is expressed (tissue specificity, *tau*) (Mank et al., 2015) or network connectivity (Marbach et al., 2012). By combining the adaptive response, as indicated by changes in gene expression, with measures of pleiotropy, we can draw conclusions regarding the influence of pleiotropy on adaptive responses. It is important to note that we do not measure the phenotype of the nonfocal traits affected by pleiotropy nor do we measure their correlation. Despite that the fitness landscape is also not known, we can use the degree of pleiotropy of selected genes to distinguish between different hypotheses about the correlation of fitness effects.

If the fitness effects of all traits affected by the focal genes are not correlated, we would expect that less pleiotropic genes show a stronger response to selection under natural and laboratory conditions. This is because these genes are less affected by the counterselection from the fitness effects of the non-focal traits. Alternatively, if the fitness effects of genes responding to temperature adaptation exhibit complete correlation, where positive and negative effects balance each other out (Martin & Lenormand, 2006), we would not expect any discernible difference in the degree of pleiotropy among the genes responding to temperature adaptation in natural and laboratory populations. Finally, if the fitness effects are more correlated either in nature or in the laboratory, we expect different outcomes. Specifically, a higher correlation of the fitness effects under natural conditions would lead to synergistic pleiotropy where the selected genes are more advantageous compared to the genes selected in the laboratory. The opposite pattern would emerge if fitness effects are more correlated in the laboratory, but we consider this an unlikely scenario.

For two different species, *D. melanogaster* and *D. simulans*, we found that the degree of pleiotropy differs for adaptive genes in the laboratory and in the wild. In both species, adaptive genes with a significant change in gene expression were more pleiotropic in natural populations than in the experimental evolution study. We conclude that this result provides strong empirical support for synergistic correlation of fitness effects in populations evolving in nature.

Materials and methods

Experimental design

Drosophila simulans and *D. melanogaster* populations were collected at the same location at the same time to minimize the influence of local adaptation in the wild on the selection response in the laboratory. This is important because the selection response to temperature may vary between populations with different temperature preadaptations. Single inseminated females were used to derive isofemale lines, which were maintained in the laboratory to distinguish both species and check for the presence of pathogens. After five generations, 10 replicate founder populations with 1,000 individuals were generated by pooling the same number of flies from each isofemale line. Half of the replicate populations were subjected to a high-temperature regime, fluctuating between 12 hr without light at 18 °C and 12 hr with light at 28 °C, mimicking the diurnal cycle. The other half of the replicate populations were exposed to a cold-temperature regime fluctuating between 12 hr at 10 °C without light and 12 hr at 20 °C with light (Figure 1).

Common garden experiments

All evolutionary replicates from both species were subjected to a common garden environment for two generations to reduce transgenerational effects. The common garden environment matched the fluctuating hot selection (18/28 °C) regime with a controlled density of 400 eggs per bottle. Mated males were separated from females under mild CO_2 anesthesia. After a 2-day recovery from the CO_2 treatment, 50 five-day-old males were flash-frozen in liquid nitrogen 8 hr after the temperature switch to 28 °C and stored at −80 °C until RNA extraction. We focused on male flies, as they exhibit much less allometric difference after the adaptation compared to females (Hsu, Jakšić, et al., 2020).

RNA extraction and sequencing

Total RNA was extracted from 50 whole-body males using the Qiagen RNeasy Universal Plus Mini kit. RNA-seq libraries were prepared on Neoprep device (software version 1.1.0.8 and protocol version 1.1.7.6, Illumina, San Diego, CA, USA) with the TrueSeq standard mRNA library kit. One hundred-nanogram RNA and default settings for an insert size of 200 bp and 15 PCR cycles were used. Libraries were randomized across library cards with identical lot number for each data set (a) *D. melanogaster* Portugal and (b) *D. simulans* Portugal. Fifty base pair reads were sequenced on the Illumina HiSeq 2500 platform.

Sequence data processing

A standardized pipeline was used for all data sets as described by Hsu, Belmouaden, et al. (2020). Briefly, the

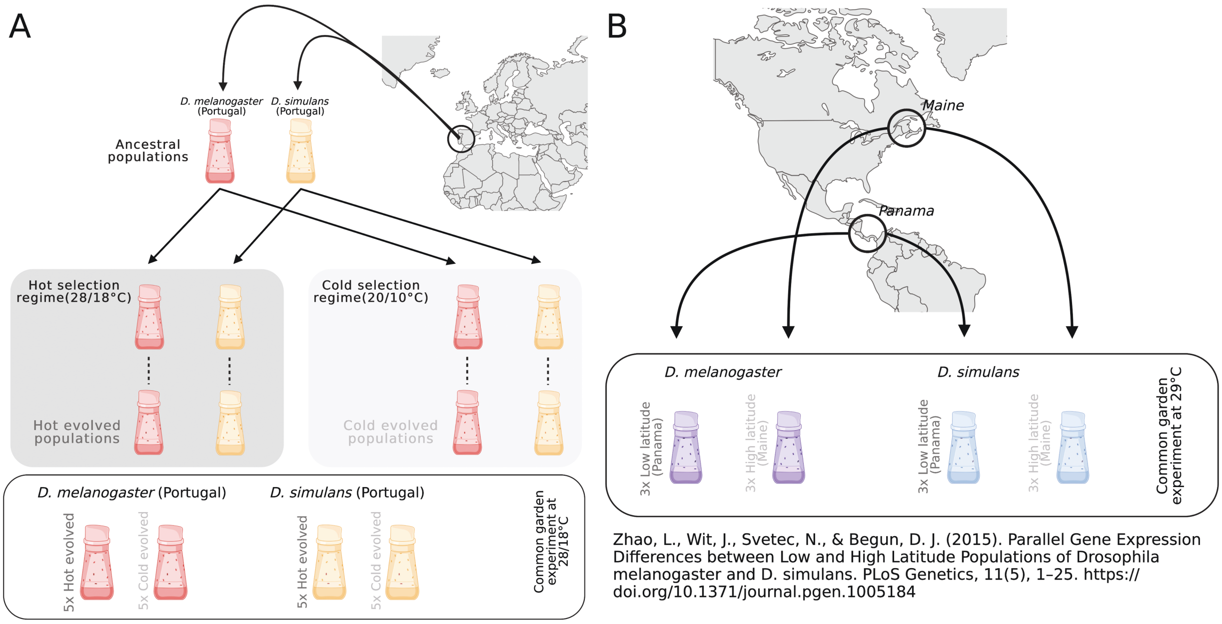

Figure 1. Study design. (A) The experimentally evolved population in a simple environment in the laboratory and (B) the natural population from the North American cline on the East Coast (Zhao et al., 2015). Created with BioRender.com.

sequenced reads were trimmed using ReadTools (version 1.5.2) (Gómez-Sánchez & Schlötterer, 2018), with a quality threshold of 20. The *D. simulans* reads were mapped using GSNAP (version 2018-03-25) (Wu & Nacu, 2010) to *D. simulans* reference genome (Palmieri et al., 2015). Since the *D. simulans* annotation was cross-referenced with *D. melanogaster* v5.49, the *D. melanogaster* reads were mapped with the same mapper to the same version from FlyBase (5.49), to avoid annotation biases when comparing the evolutionary response between species. The following parameters were used for mapping all data sets: -A: SAM, -k: 15, -N: 1, -m: 0.08. mRNA quality was checked by controlling for 3′-bias using RSeQC (Wang et al., 2012). Rsubread (version 2.2.2) (Liao et al., 2019) was used for quantifying reads aligned to mRNA along with the annotation version 5.49 for the *D. melanogaster* genome (Hoskins et al., 2007), and exons along with a matching annotation file for *D. simulans*. The publicly available data (Zhao et al., 2015) were processed with the same pipeline to avoid technical biases during downstream analysis.

Statistical analysis

Each of the four count tables was filtered for genes that expressed ≥1 CPM in at least one sample, removing lowly expressed genes. For *D. melanogaster*, 11,069 and 10,467 of 13,968 annotated genes remained after this filtering for the experimentally evolved population and the natural populations, respectively. For *D. simulans*, 10,922 and 11,004 of 13,262 annotated genes remained for the experimentally evolved populations and the natural populations, respectively. Differential expression analysis was carried out with edgeR (3.30.3) (Robinson et al., 2009).

To evaluate the evolutionary expression response for each gene, a linear model was fitted using the function glmFit() in edgeR, where normalized gene expression for each gene is the response variable and the evolutionary regime is the explanatory variable:

$$\text{Expression} = \text{evolution} + \varepsilon.$$

Contrasts between hot- and cold-evolved samples for experimentally evolved populations, and high and low latitudes for populations in nature (Zhao et al., 2015) were performed using the function glmLRT(). We accounted for multiple testing by using Benjamini and Hochberg's FDR (Benjamini & Hochberg, 1995). The magnitude of the evolutionary expression change was determined by the $\log_2$ scaled fold change ($\log_2$FC). As the flies were measured in a common garden, we can rule out that the expression differences are due to plasticity. Rather, they reflect genetic differences between the populations. Since we used independently evolved replicate populations, we consider consistent changes among replicates in the same direction as evidence for selection-driven changes in gene expression, rather than the consequence of genetic drift. Natural populations have large population sizes, which makes the influence of genetic drift less likely; hence, we follow a common practice and consider significant differences in gene expression the result of selection, even without the analysis of replicate populations. Consistent with nonrandom changes in gene expression, we observed significant enrichment of some gene ontology (GO) categories among genes classified as selected.

GO enrichment analysis of significant differentially expressed genes was used to classify the functional implications of gene expression evolution. We used the package topGO (Alexa & Rahnenfuhrer, 2020) with the "Weighted01" algorithm, which accounts for the hierarchy of GO terms.

Pleiotropy indexes

Two independent pleiotropy indexes were used, tissue specificity (*tau*) and network connectivity. The implicit, but realistic, assumption of our measure of pleiotropy is that a change in gene expression will have phenotypic consequences.

Tau describes the specificity of gene expression across different tissues. It was calculated based on tissue-specific expression of adult males from Flyatlas2 (Krause et al., 2022; Leader et al., 2018).

$$Tau = \frac{\sum_i \left[1 - \frac{\text{gene} \quad \text{expression}_i}{\text{gene} \quad \text{expression}_{\max}}\right]}{N-1}$$

where N is the number of tissues analyzed (Mank et al., 2015). If a gene is expressed in a single tissue, *tau* will equal 1, while it equals 0 when a gene is expressed at the same level across all tissues. The relationship between *tau* and pleiotropy is based on the idea that genes expressed in many tissues are more likely to affect multiple traits than genes expressed in fewer tissues. Hence, we used 1-*tau* to indicate the pleiotropic effect of a gene. *Tau* has been shown to correlate with QTL-based pleiotropy measures (Watanabe et al., 2019) and is an established proxy of pleiotropy (Mank et al., 2015).

Network connectivity represents the sum of all edges to a gene in an integrative regulatory network of *D. melanogaster*. The network was reconstructed from both functional and physical regulatory interaction, using machine learning (Marbach et al., 2012). The physical interactions are based on conserved transcription factor site motives and experimentally determined transcription factor bindings. The functional regulatory interaction consists of various data, related to chromatin modification and gene regulation (Marbach et al., 2012). Network connectivity is widely used as a proxy for pleiotropy (Papakostas et al., 2014; Rennison & Peichel, 2022).

Both proxies are L-distributed (Supplementary Figure 3) as other estimates of pleiotropy (reviewed in Wagner & Zhang, 2011) and are highly correlated (Spearman's *rho* = 0.512; p-value < 2.2e-16). It is remarkable that the two pleiotropy estimates exhibit a high correlation, despite being based on two entirely different measures. This suggests that the pleiotropy estimates are robust and not substantially impacted by the environmental heterogeneity, as the data for both estimates were acquired without enforcing the same environmental conditions. This underscores the value of using multiple pleiotropy measures to assess the robustness of our findings.

Results

Temperature-mediated gene expression evolution

We used gene expression from two *Drosophila* species, *D. melanogaster* and *D. simulans*, to study adaptation to different temperature regimes. For both species, two populations from a North American cline on the East Coast and replicated populations evolved to hot- and cold-temperature regimes in the laboratory were analyzed (Figure 1). The gene expression was measured in two common gardens, one for the natural populations (Zhao et al., 2015) and another one for the laboratory populations (see Materials and methods; Figure 1). Principal component analyses of the experimentally evolved population pairs indicated a clear separation of the populations evolved at different temperature regimes (Supplementary Figures 1 and 2). One hundred and eighty-four genes were differentially expressed in *D. melanogaster* (77 genes were more highly expressed in the hot evolved and 107 genes were expressed at a higher level in the cold evolved) and 130 in *D. simulans* (68 were more expressed in the hot evolved and 62 were more expressed in the cold evolved) in a common garden temperature of fluctuating 18/28 °C. Considerably more genes were differentially expressed between the natural populations. The gene expression of high- and low-latitude populations differed significantly for 812 genes in *D. melanogaster* (286 genes were expressed at higher levels in the low-latitude populations and 526 genes were more expressed in the high-latitude populations) in a common garden temperature of 29 °C. In *D. simulans*, 663 genes evolved significant expression differences (403 genes were more highly expressed in the low-latitude populations, and for 260 genes, the expression level was higher in the high-latitude populations).

Genes with low to intermediate pleiotropy experience the strongest response to selection

Gene expression differences between population pairs, which evolved at divergent temperature regimes, provide a genome-wide estimate of adaptive responses. Given that estimates of pleiotropy are available for most genes, we can investigate the influence of pleiotropy on adaptive gene expression changes while assuming that these measures of pleiotropy are independent of the environmental conditions under which they were inferred. We used two measures of pleiotropy, tissue specificity (Mank et al., 2015) and connectivity (Marbach et al., 2012), to test for a correlation between the degree of pleiotropy and the magnitude of gene expression change ($\log_2$FC).

In all four population pairs, we detected a negative correlation between the expression change and pleiotropy, independent of whether we used tissue specificity (1-*tau*) or connectivity (Table 1, Figure 2, Supplementary Figures 4 and 5). We caution, however, that the relationship between pleiotropy and gene expression change may not be linear. Previous work suggested that genes with intermediate levels of pleiotropy respond most to selection (Frachon et al., 2017; Wang et al., 2010). This trend can also be seen in all four population pairs, the strongest gene expression change was seen for low to intermediate values (Figure 2). Focusing on genes with a significant change in gene expression, provided further support for the importance of genes with intermediate pleiotropic effects. In all four contrasts, genes with low to intermediate effects are overrepresented compared to background genes (i.e., all genes with available pleiotropy measurements) (Figure 3). This pattern confirms that genes with a higher degree of pleiotropy are less likely to contribute to adaptation

Table 1. Negative correlation between pleiotropy and adaptive response. Spearman's correlation coefficients (rho) and p-values between the two pleiotropic proxies and $\log_2$FC in the four population pair contrasts. p-Values are corrected for multiple testing with Benjamini and Hochberg's FDR (Benjamini & Hochberg, 1995).

Population pair contrasted	1-*tau*	Connectivity
D. melanogaster Panama–Maine	rho −0.297 (p_{adj} = 1.8e-207)	rho −0.217 (p_{adj} = 6.6e-98)
D. simulans Panama–Maine	rho −0.138 (p_{adj} = 7.1e-46)	rho −0.140 (p_{adj} = 1.2e-42)
D. melanogaster Hot–Cold	rho −0.262 (p_{adj} = 3.2e-170)	rho −0.196 (p_{adj} = 6.6e-84)
D. simulans Hot–Cold	rho −0.168 (p_{adj} = 3.1e-67)	rho −0.153 (p_{adj} = 9.9e-51)

and the same pattern is seen for temperature adaptation in the laboratory and natural populations.

A higher degree of pleiotropy for differentially expressed genes in natural environments

If the fitness effects are not correlated across genes, we would expect that less pleiotropic genes respond more to selection in both data sets from laboratory and natural populations. In the case of fully correlated fitness effects where positive and negative effects cancel out, no difference should be observed in the degree of pleiotropy for the genes responding to temperature adaptation in both data sets. However, we find that in natural population genes with significant differences in gene expression between populations from the two temperature regimes are on average more pleiotropic (1-*tau*) (Figure 4). This pattern is fully consistent in both species, *D. simulans* and *D. melanogaster* (*D. melanogaster p* = 3.4e-05 and *D. simulans p* = 6.4e-04, two-sided Wilcoxon rank sum test). The same trend was seen for connectivity, although not significant in *D. simulans* (Supplementary Figure 6) (*D. melanogaster p* = .0074 and *D. simulans p* = .15, two-sided Wilcoxon rank sum test). Combining the *p*-values for both measures of pleiotropy with Stouffer's method (Stouffer et al., 1949), the pattern of higher pleiotropy in natural populations was confirmed (*D. melanogaster p* = 2.8e-06 and *D. simulans p* = .0013). This suggests that fitness effects are more correlated in natural environments than in the laboratory.

Functional response to temperature selection

An enrichment analysis of the differentially expressed genes identified several biological pathways that have presumably

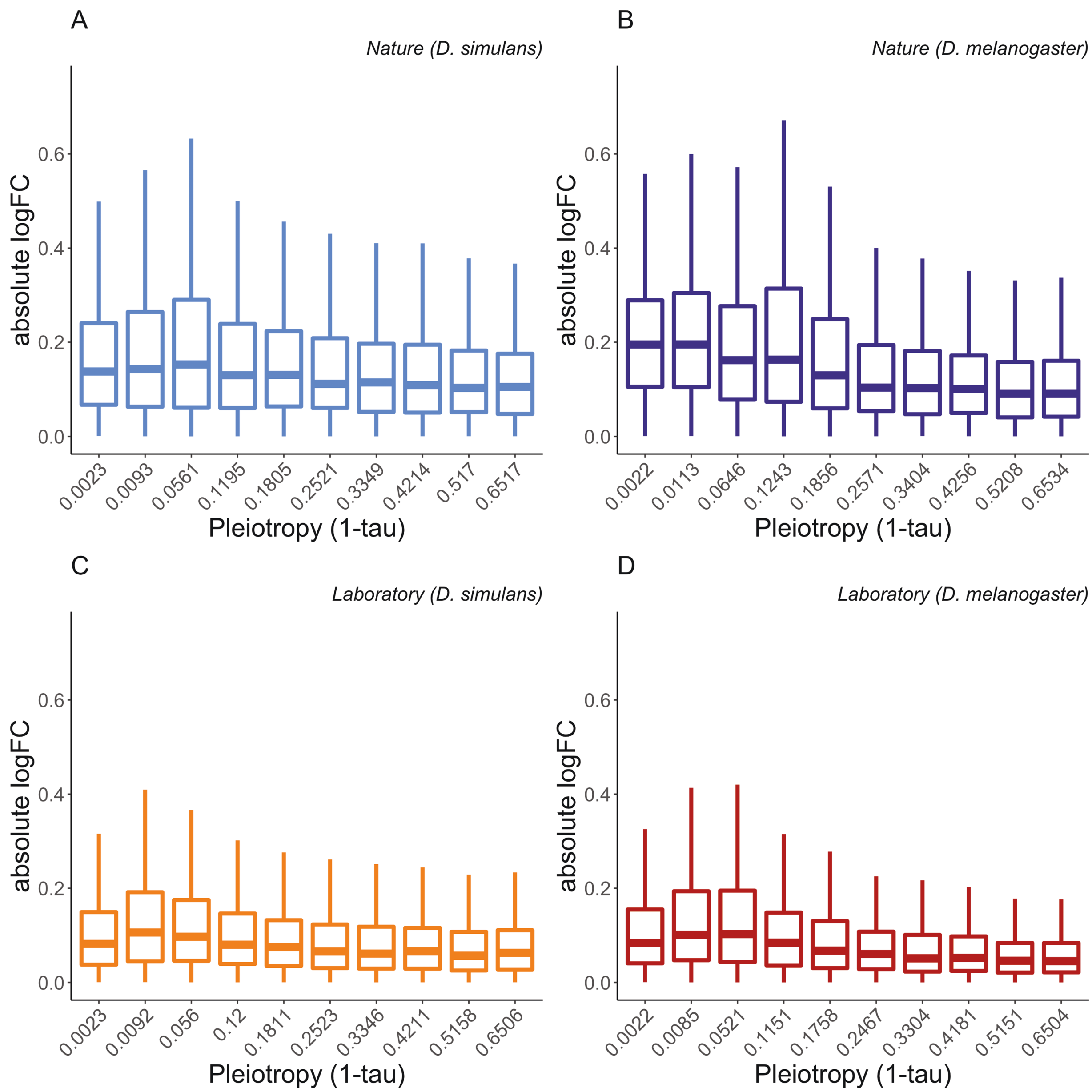

Figure 2. Magnitude of change is negatively correlated with pleiotropy. Absolute $\log_2$ fold change of all genes with information of pleiotropy (1-*tau*) plotted against 10 equal-sized bins of pleiotropy (1-*tau*). The average of 1-*tau* in each bin is given on the x-axes. The four panels in different colors represent the four contrasts of population pairs: (A) *D. simulans* clinal populations, (B) *D. melanogaster* clinal populations, (C) *D. simulans* experimentally evolved populations, and (D) *D. melanogaster* experimentally evolved populations. Outliers were excluded for visualization.

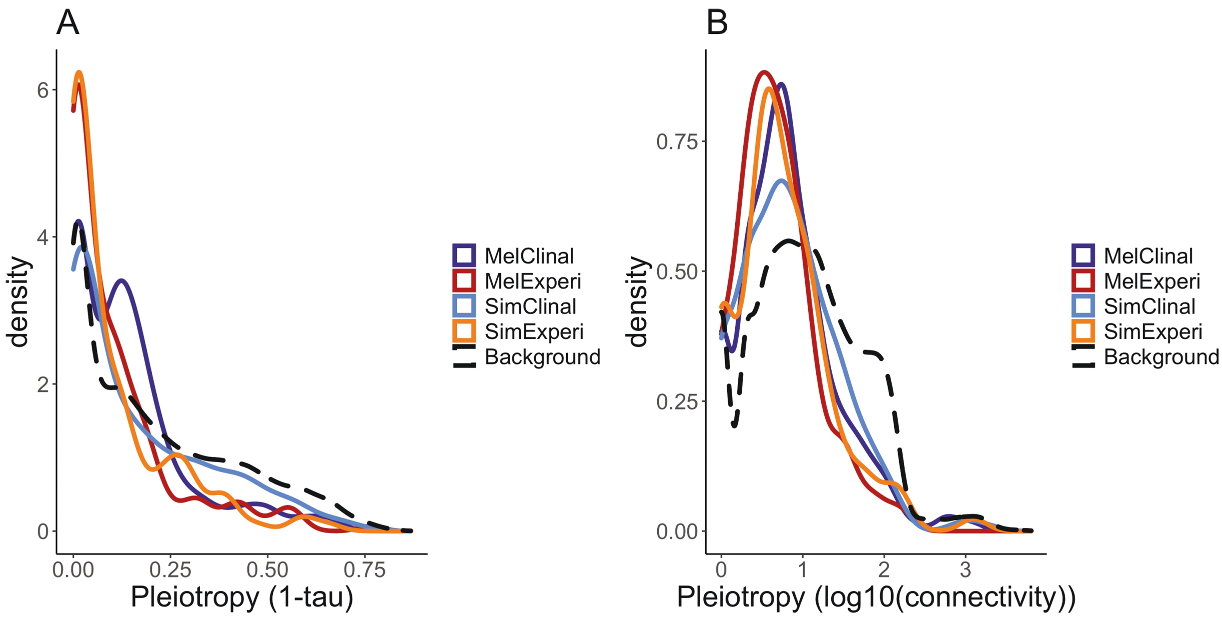

Figure 3. More genes with low to intermediate degrees of pleiotropy experience significant gene expression differences. The lines represent kernel density estimates of genes with significant differences in gene expression, plotted for two different measures of pleiotropy: (A) tissue specificity and (B) connectivity. Each population pair is indicated in a different color. The dashed black line represents the background of all genes with pleiotropy measurements.

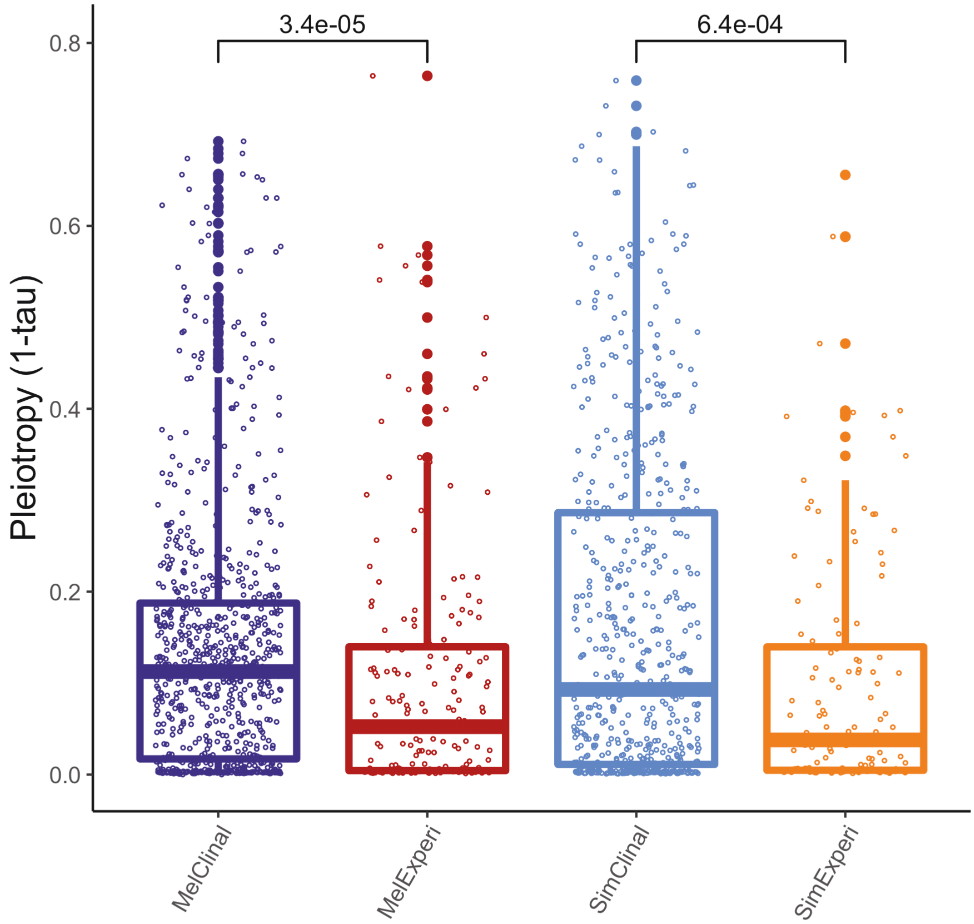

Figure 4. Higher pleiotropy for genes with significant expression differences between populations from different temperature regimes in the wild than in the laboratory. Each open dot indicates the measure of pleiotropy (1-*tau*) for a gene, which is significantly different expressed (FDR < 0.05) in the contrast of population pairs from different temperature regimes. *p*-Values from two-tailed Wilcoxon rank sum test.

functionally diverged between the population pairs. Proteolysis was not only the most significant GO term in the experimental populations but also enriched in the two clinal contrasts (Supplementary Data 1–4). In the clinal *D. melanogaster* comparison, many GO terms related to neuronal signaling were enriched, which was not apparent in the clinal *D. simulans* contrast—only the GO term "response to nicotine" was significant. In the *D. melanogaster* experimental population contrast, the GO terms "neuropeptide signaling" and "synaptic target attraction" were marginally significant. Experimental populations of *D. simulans* showed significant enrichment for genes related to sphingolipid and ceramide GO terms. This may not only indicate some changes in neuronal signaling but may also relate to changes in the membrane composition. Furthermore, the GO term "catecholamine biosynthetic process" points toward the evolution of neuronal signaling in the experimental *D. simulans* populations. Nevertheless, the strong enrichment of dopamine-related signaling, which was detected in experimentally evolved populations with ancestry from Florida (Jaksic et al., 2020), was not seen in this study. Interestingly, in natural and experimental populations, more significant GO categories were detected in *D. melanogaster* than in *D. simulans*.

Discussion

This study leverages a very special data set encompassing populations that have adapted to distinct temperature regimes in different settings. By harnessing these data, we investigated the role of pleiotropy for adaptive gene expression changes.

Consistent with the expectations for uncorrelated fitness effects (Fisher's model), we detected a negative correlation between pleiotropy and the level of evolved gene expression change (Table 1, Figure 2, Supplementary Figures 4 and 5). This observation is consistent with a previous study on protein expression evolution (Papakostas et al., 2014) and the observation that mutations affecting the expression pattern of more genes have larger fitness costs (Zande et al., 2022).

On the other hand, the comparison of adaptive responses under the two settings, nature and laboratory, highlights another intriguing aspect of pleiotropy, which is frequently overlooked. In two studied *Drosophila* species, we observed that genes exhibiting significant differences in gene expression between the two temperature regimes had a higher degree of pleiotropy in clinal populations compared to laboratory populations (Figure 4 and Supplementary Figure 6). These findings provide evidence to support the notion that fitness effects in natural populations are more correlated than in the laboratory. Consequently, when standing genetic variation is filtered for correlated fitness effects in the wild, in a new environment (i.e., in the laboratory), these fitness effects are no longer correlated, which results in a cost of pleiotropy for standing genetic variation.

Alternative explanations

The founder populations of the experimental evolution studies originated from Portugal, while the clinal populations were collected on the US East Coast. It may be possible that these samples are genetically differentiated such that different contributing alleles were segregating in the populations or the same alleles had different starting frequencies. While both factors can contribute to use of alternative genes for adaptation, no systematic difference in the degrees of pleiotropy is expected under this scenario. Furthermore, the highly consistent signature of more pleiotropic effects in clinal populations of *D. melanogaster* and *D. simulans* makes stochastic differences between the natural and laboratory environments a less likely explanation for the observed patterns.

Estimates of the degree of pleiotropy

We relied on two widely used estimates for the degree of pleiotropy: tissue-specific expression and connectivity (Fraïsse et al., 2019; Mank et al., 2015; Marbach et al., 2012; Papakostas et al., 2014; Rennison & Peichel, 2022; Watanabe et al., 2019). Because we used whole-body gene expression data, gene expression changes in opposite direction in different tissues can be averaged out. This averaging effect will be more pronounced for genes with broad expression than for genes expressed in a few tissues. This implies that an adaptive gene expression change may be less likely to be detected with whole-body RNA-seq data. This would result in fewer genes with a significant gene expression change if they are expressed in many tissues. Hence, this pattern is consistent with a negative correlation between gene expression change and pleiotropy (i.e., number of tissues). Nevertheless, given the similarity of our results for the two estimators of pleiotropy, we do not consider that this potential issue has influenced our analysis. More importantly, the comparison of natural environment and laboratory environment is not affected because in both cases whole-body gene expression data were used.

Conclusion

Our study provides compelling empirical evidence for the dual role of pleiotropy for adaptation. The lower contribution of pleiotropic genes to the adaptive response in laboratory and natural populations is fully consistent with the retarding effect of pleiotropy caused by noncorrelated fitness effects of nonfocal traits. The more subtle effect of pleiotropy enhancing fitness effects could only be detected in our experimental design. We compared the selection response in natural populations, with many correlated fitness effects to populations, which evolved in the laboratory where the correlation of fitness effects is much less pronounced. We propose that our understanding of polygenic adaptation crucially depends on more insights about the dual role of pleiotropy for selection responses. Further studies on the evolutionary response with different correlations of fitness effects can characterize the relative importance of correlated fitness effects for adaptation.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Sequenced reads from this study will be made available upon publication on the European Sequence Read Archive (https://www.ebi.ac.uk/ena), under the accession number PRJEB57451. Read count tables from all populations and scripts for analysis will be available on the GitHub repository for this study upon publication (https://github.com/DagnyAsta/PleiotropyAndCorrelatedFitnessEffects.git).

Author contributions

D.A.V.T. and C.S. conceived the study and wrote the manuscript. D.A.V.T. performed the analysis. V.N. prepared the

RNA-seq libraries published in this study and supervised common garden experiments and the maintenance of the experimental evolution experiments.

Conflict of interest: The authors declare no conflict of interest.

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