

Genetic Analysis of Micro-environmental Plasticity in *Drosophila melanogaster*

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ABSTRACT: Classical quantitative genetic models recognize the potential for genotype by environment interaction, whereby different genotypes have different plastic response to changes in macro-environmental conditions. Recently, it has been recognized that micro-environmental plasticity ('residual' variance) may also have a genetic component. This study utilized the *Drosophila* genetic reference panel (DGRP) to estimate the genetic variance of micro-environmental plasticity for two quantitative traits, chill coma recovery and startle response. The strongly controlled experimental design allowed the genetic parameters to be accurately estimated. Both traits had high broad sense heritabilities (from 0.51 to 0.77), of the same order as the mean for startle response and even greater than the mean for chill coma recovery. Genome wide association analyses identified molecular variants (from 15 to 31 depending on the sex and the trait) associated with micro-environmental plasticity. These findings provide the opportunity of selecting for micro-environmental plasticity.

Keywords:

Environmental plasticity

Selection

Genetic parameters

DGRP

GWAS

Introduction

The term 'environmental plasticity' is used to describe changes in the mean of a quantitative trait phenotype associated with a defined change of macro-environmental conditions as well as responses to micro-environmental variation that cannot be attributed to a specific perturbation. Environmental plasticity can have a genetic component. In the case of variation among macro-environments (e.g. temperature, level of nutrition), genetic variation in plasticity gives rise to genotype by environment interaction. Genotype by environment interaction for a quantitative trait can be readily quantified by rearing the same genotypes in different macro-environments. Less well known and readily quantified is genetic variation for micro-environmental plasticity, which requires rearing the same genotypes within the same macro-environment, and determining within the residuals, or environmental, variance differs among genotypes. An emerging body of evidence indicates that there is indeed genetic variance for the magnitude of micro-environmental variance (Hill and Mulder (2010)). The phenomenon has been documented for several traits in chickens (Rowe et al. (2006)), rabbits

(Ibáñez-Escriche et al. (2008)), snails (Ros et al. (2004)), pigs (Sorensen and Waagepetersen (2003)), sheep (SanCristobal-Gaudy et al. (1998)) and flies (Mackay and Lyman (2005)). The existence of genetic variance for micro-environmental plasticity has implications for selective breeding programmes. One could select animals for decreased micro-environmental plasticity so that they to increase the uniformity of the final product. There are two major challenges. First, micro-environmental plasticity is often not normally distributed which may cause problems when linear models are applied. Second, a strongly controlled experimental design is needed to precisely quantify any genetic component for variation in environmental plasticity, which is difficult to realize in non-model organisms. In fact, much of the work designed to provide evidence for genetic control of micro-environmental variation was based on analysis of field data. This required implementation of complex hierarchical models to account for the structure of the data. Results are sensitive to model assumptions and the distribution of the data and can be misleading (Yang et al. (2011)). Thus, we used the *Drosophila melanogaster* Genetic Reference Panel (DGRP), a collection of 205 inbred fly lines with complete genome sequence, to evaluate which metric should be used to measure micro-environmental plasticity and to estimate the heritability of environmental plasticity for two traits, chill coma recovery and startle response (Mackay et al. (2012)). Furthermore, we also performed genome wide association (GWA) analyses to map molecular variants associated with micro-environmental plasticity for the two traits.

Materials and Methods

Data. The 205 DGRP lines were constructed by 20 generations of full-sib mating isofemale lines collected from the Raleigh, NC, USA population. Time to recover from a chill-induced coma for 174 lines with two replicates per line (N=34,653 of which N=17,382 for females and N=17,271 for males). Startle response was measured for 201 lines with two replicates per line (N=16,214 of which N=8,114 for females and N=8,100 for males). These phenotypes were accurately measured in the same controlled macro-environment so different phenotypes experienced only different micro-environments.

Estimating the genetic variance. The statistical analysis was performed on the two sexes separately. A linear model including only the intercept ($Y = \mu + E$ where μ is the overall mean and E is the within replicate micro-

environmental variance) was fitted to the data for each line and replicate separately and the residual standard deviation (σ_e) was calculated from the mean squared error. The coefficient of environmental variation (CV_e) was also calculated as $\sigma_e/\mu_{lr} \times 100$ where μ_{lr} is the mean phenotype of the line within the replicate. Then, a new dataset (two records per line, one from replicate 1 and one from replicate 2) was constructed with these two metrics of micro-environmental plasticity. A random effect ANOVA ($Y = \mu + L + E$ where Y is σ_e , μ is the overall mean, L is the random effect of line and E is the variance in σ_e between replicates) was then fitted to the new dataset to estimate the genetic variance ($\sigma_g^2 = \sigma_{line}^2$) and the broad sense heritability ($H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ee}^2)$ where σ_{ee}^2 represents the environmental variance of environmental plasticity) for micro-environmental plasticity. The metric of micro-environmental variation was also analyzed after log-transformation.

GWA Study. The statistical analysis was performed on the two sexes separately. To create the data set for the GWA study (one record per line), the data was analyzed across the two replicates and a random effect model ($Y = \mu + R + E$ where μ is the overall mean, R is the random effect of the replicate and E is the environmental variance) was fitted to each line separately. The residual standard deviation (σ_e) was extracted and the coefficient of environmental variation (CV_e) was also calculated as $\sigma_e/\mu_l \times 100$ where μ_l is the mean phenotype of the line. A GWA study was then performed for each trait on the log-transformed σ_e using a mixed model accounting for the relationship among the lines, implemented in the DGRP website (Mackay et al. (2012)).

Results and Discussion

Evaluation of the metrics. The two metrics of micro-environmental variance considered (σ_e and CV_e) showed a very skewed distribution which became approximately normally distributed when log-transformed. Figure 1 shows the distribution of σ_e before and after the log-transformation in females for chill coma recovery. This trend is very similar across replicates, sexes, traits and metrics.

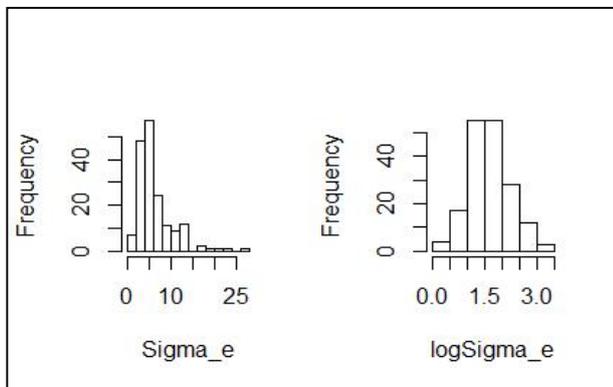


Figure 1. Distribution of σ_e in females for chill coma recovery without (left) and with (right) log-transformation.

Furthermore, there was no dependency of σ_e on the mean (assessed using an informal test) and, therefore, σ_e and not CV_e for further analyses.

Estimating the genetic variance. The results for chill coma recovery and startle response are presented in Table 1 and Table 2, respectively.

Table 1. Variance components for micro-environmental plasticity measured as σ_e for chill coma recovery in females (F) and males (M).

	σ_e				
Sex	σ_g^2	σ_{ee}^2	H^2 ³	F.value ⁴	p.value ⁵
F	14.114	4.139	0.773		
M	10.772	5.109	0.678		
log σ_e					
F	0.281	0.084	0.770	7.697	0
M	0.263	0.091	0.743	6.789	0

¹Genetic variance of environmental plasticity

²Environmental variance of environmental plasticity

³Broad sense heritability

^{4,5} F test and relative p-value to test the significance of the genetic variance

Table 2. Variance components for micro-environmental plasticity measured as σ_e for startle response in females (F) and males (M).

	σ_e				
Sex	σ_g^2	σ_{ee}^2	H^2 ³	F.value ⁴	p.value ⁵
F	1.348	1.325	0.504		
M	1.353	1.248	0.520		
log σ_e					
F	0.059	0.057	0.509	3.080	4×10^{-15}
M	0.056	0.052	0.519	3.169	9×10^{-16}

¹Genetic variance of environmental plasticity

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^{4,5} F test and relative p-value to test the significance of the genetic variance

These results are remarkable. They confirm that micro-environmental plasticity is under genetic control and that genetic variance of micro-environmental plasticity is not only significant but of large magnitude. Indeed, the broad sense heritabilities of log σ_e ranged from 0.51 to 0.77 for the two traits. It is worth noting that the broad sense heritability of micro-environmental plasticity for chill coma recovery is much higher than is for the mean (~ 0.75 vs 0.43 averaged over the two sexes (Mackay et al. (2012))). The broad sense heritability of micro-environmental plasticity for startle response, the H^2 of environmental plasticity is of the same magnitude as for the mean (~ 0.51 vs 0.56 (Ober et al. (2012))). The heritabilities obtained in the present study are very much higher than the ones that can be found in literature using non-model organism and field data, which range from 0.00 to 0.20 (Hill and Mulder (2010)). One possible reason is that broad sense heritability was estimated here, whereas the previous estimates were of narrow sense heritability. Epistatic genetic variance could thus account for these differences. This may also be explained by the use of highly parameterized models to model the heterogeneity of the residual variance when using

field data which provide less reliable estimates (Yang et al. (2011)).

GWA Study. GWA analyses of micro-environmental variance identified 15 and 29 variants for females and males respectively associated ($P < 10^{-5}$) with environmental plasticity for chill coma recovery. For startle response, 20 and 31 variants for females and males respectively were associated ($P < 10^{-5}$) with environmental plasticity. The genes affected by these variants were enriched for processes and pathways related to the biology of those traits. Interestingly, different variants and genes were associated with micro-environmental plasticity of the two traits. The hypothesis that a common process affects micro-environmental plasticity irrespective of the trait is thus not supported by these data.

Conclusion

Micro-environmental plasticity, which is best measured by using the log-transformation of v_e , is shown to be under strong genetic control in *D. melanogaster*. This leads to the opportunity of selecting for decreased micro-environmental plasticity to favor individuals giving a more uniform final product. High values of broad sense heritabilities of micro-environmental variance suggest that good genetic progress could be even higher than for mean performance. There is almost no overlap among variants associated with micro-environmental plasticity and variants associated with mean performance (Mackay et al. (2012)) for both the traits analyzed indicating that selection can be performed on both components. The GWA analyses have provided candidate genes for future validation using genetic resources available in *D. melanogaster*.

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