Genetic analysis of environmental variation of cold tolerance in *Drosophila melanogaster*

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The genetics of environmental variation

- Heritable factors and environmental variation do not act independently because heritable variation controls the expression of environmental variation [1-2]. However, the underlying genetic mechanisms controlling levels of environmental variation (Fig. 1) are poorly understood.
- Here we investigate cold tolerance measured as the Critical Thermal Minimum (CT_{min}, Fig. 2). We associate variation in CT_{min} with genomic variation by genomic prediction to determine the genetic architecture controlling different sources of environmental variation, and how they are connected.
- Additionally, we investigate the genetic mechanisms behind inherent cold tolerance across a thermal developmental gradient.

We propose four sources of environmental variation:

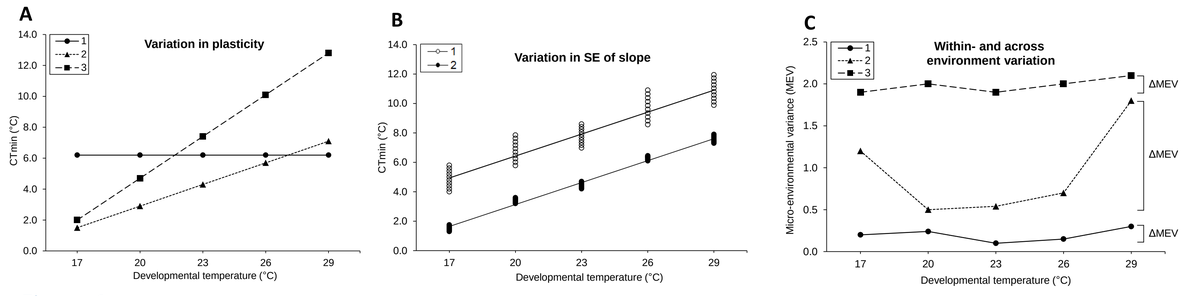

Figure 1. Conceptual illustrations of the four sources of environmental variation in hypothetical lines (1, 2 or 3). **A.** plasticity; a given genotype's ability to produce a range of phenotypes in response to different environments. **B.** variation of the plastic response; measure of the extent to which the same genotype can produce the same plastic response across environments. In a linear relationship this is calculated as SE of slope. **C.** micro-environmental variances; (MEVs) [2] and mean MEV measures the extent to which the same phenotype can be produced from the same genotype within the same environment. Right hand side of B shows heterogeneity of micro-environmental variation (ΔMEV) across environments. This measures the extent to which the expression of phenotypic variation within an environment is constant across the range of environments.

Assessing cold tolerance

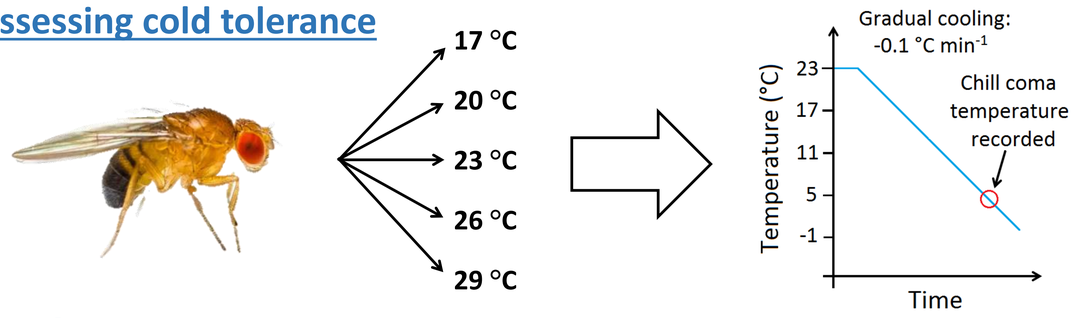

Figure 2. Outline of the experimental procedure of assessing cold tolerance. Flies were reared at five different developmental temperature. After eclosion the flies were assessed for CT_{min} measured as the temperature at which no movement is observed after a period of gradual cooling.

- 166 fully inbred lines from the *Drosophila* Genetic Reference Panel (DGRP) [3] were reared at five developmental temperatures.
- 10 males from each line from each developmental temperature were gradually cooled in a water bath. CT_{min} is the temperature at which the fly enter chill coma.
- We measured the four types of environmental variation as the slope from a linear regression (plasticity, Fig. 1A), the standard error of the slope (Fig. 1B), mean MEV and ΔMEV (Fig. 1C), and we computed broad sense heritability (H^2) for all traits (Table 1).
- We used a Genomic Feature Best Linear Unbiased Prediction (GFBLUP) [4] model to search for potential causative gene ontologies (GOs). The predictive ability (PA) of each GO was computed as the correlation between the observed genetic value and predicted genetic value using tenfold cross validation (Table 1).
- GOs were decomposed to gene level [5]. We looked at genes that explain >10% of the variation within each GO (Table 2). So far, we have investigated the top 5 GOs with the highest PA from each trait.

Table 1. Broad sense heritability (H^2) and predictive ability (PA) for CT_{min} at five developmental temperatures, slope, SE of slope, mean MEV, ΔMEV, and MEVs at all temperatures. We also calculated additive SNP-narrow sense heritability (h^2_{SNP}) as the proportion of phenotypic variation captured by the common SNPs. They were similar to H^2 for all traits.

Trait	H2	PA
CT_{min} at 17 °C	0.43	0.27
CT_{min} at 20 °C	0.51	0.26
CT_{min} at 23 °C	0.49	0.13
CT_{min} at 26 °C	0.50	0.06
CT_{min} at 29 °C	0.47	-0.07
Slope	0.70	-0.08
Slope SE	0.32	0.19
Mean MEV	0.27	0.29
ΔMEV	0.18	0.28
MEV 17 °C	0.07	0.39
MEV 20 °C	0.18	0.14
MEV 23 °C	0.12	0.36
MEV 26 °C	0.14	0.03
MEV 29 °C	0.14	0.30

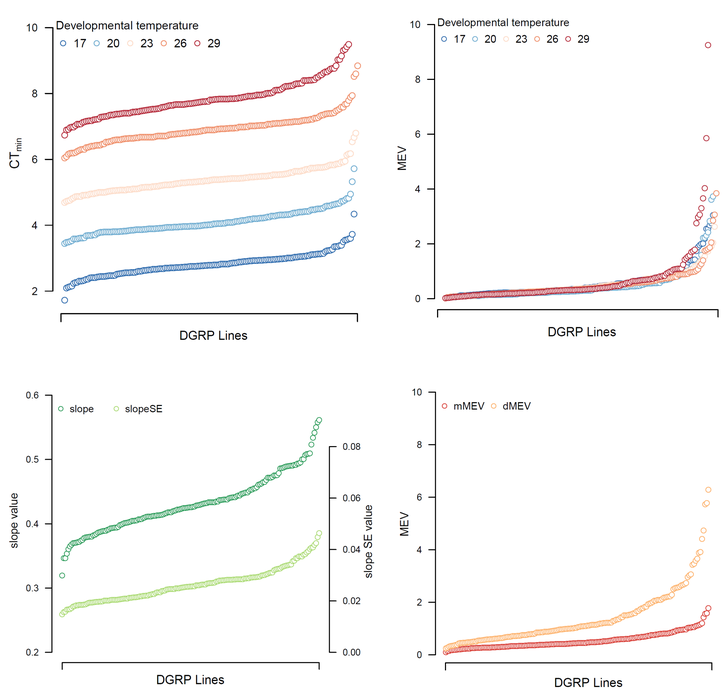

Figure 2. Distributions of CTmin (top left), and MEV (top right) for all developmental temperatures as well as mean MEV and ΔMEV (bottom right) and slope and slope SE (bottom left) sorted for the DGRP lines showing line effects. Each trait is sorted by effect, thus observations of several traits within each chart do not necessarily represent the same DGRP line.

Little or no overlap in causative genes suggest separate genetic mechanisms depending on the environment

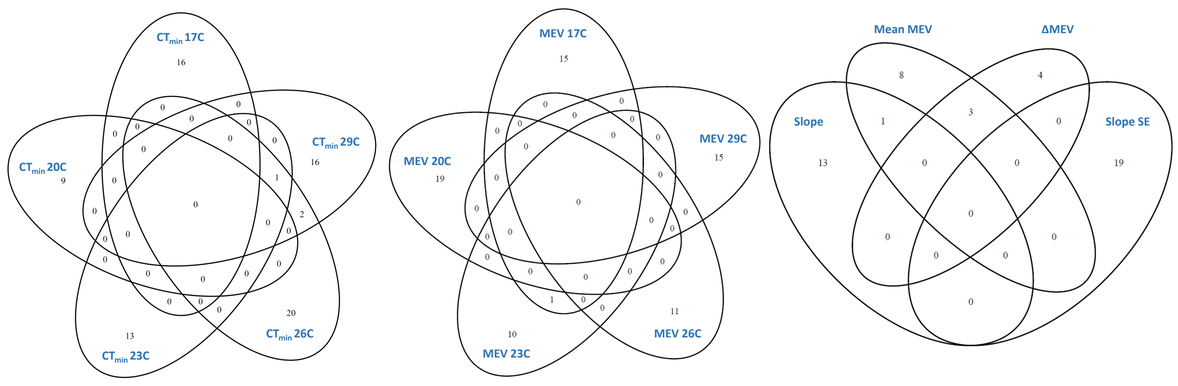

Figure 3. Venn diagrams showing overlap in causative genes in 3 groups (from left): CT_{min} and MEV at the five developmental temperatures, and between slope, slope SE, mean MEV and ΔMEV. Only genes that explain >10% of the variation within each of the top 5 GOs with the highest PA for each trait is considered here.

Table 2. Genes explaining most variation within GOs with the highest PA of selected trait. The predictive ability of GOs, and the percent of variation within the GO explained by the gene is shown. Notes on functions obtained from FlyBase.org and scientific literature.

Trait	Gene ontology	PA	Gene	Function	% Variation
CT_{min} 17 °C	GO:0006626	0.37	*Hsp60D*	Protein refolding and heat protection	24.1
CT_{min} 29 °C	GO:0007306	0.28	*nudel*	Peptidase activity	38.4
MEV 17 °C	GO:0016333	0.77	*Egfr*	Cell fate commitment and development	41.2
MEV 29 °C	GO:0030674	0.53	*phyllopod*	Regulation of protein degradation	57.3
Mean MEV	GO:0030674	0.53	*Sgt1*	Involved in chaperone binding	33.4
ΔMEV	GO:0001708	0.62	*tll*	Cell fate commitment and development	29.0
Slope	GO:0004843	0.34	*calypso*	Chromatin binding and silencing	29.5
Slope SE	GO:0016180	0.49	*IntS11*	Integrator gene responsible for proper gene expression	29.8

Conclusions

- We found that causal genes were temperature dependent, suggesting environment specific mechanisms acting in CT_{min}, as well as MEVs and sources of variation (slope, slope SE, mean MEV, and ΔMEV).
- We found that the *Hsp60D* gene was involved with cold tolerance at low temperatures, whereas at high temperatures genes involved in regulating protein activity and degradation were of higher importance.
- Genes related to slope, slope SE and mean MEV were all involved in regulating chromatin, chaperones or integrator processes, all of which have been suggested to be of great importance in epigenetic regulation, and thus perhaps in overall plasticity of a phenotype.

References [1] Ayroles, et al. (2015). *Proc. Natl. Acad. Sci*. 112, 6706–6711. [2] Morgante, et al. (2015). *Sci. Rep*. 5, 9785. [3] Mackay, et al. (2012). *Nature*, 482, 173–178. [4] Edwards, et al. (2016). *Genetics*. 116, 187161. [5] Rohde, et al. (2016). *Int. J. Genomics.* 1-11.