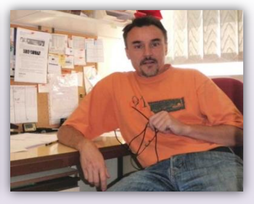
Targeted transcriptomic analysis of diapause development in larvae of the drosophilid fly, *Chymomyza costata*.



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Introduction

Diapause is a developmental alternative to direct ontogeny in many invertebrates. Its primary adaptive meaning is to secure survival over unfavourable seasons in a state of developmental arrest, which is usually accompanied by metabolic suppression and enhanced tolerance to environmental stressors.

Insect diapause development consists of a sequence of several ecophysiological phases, which are poorly characterized in biochemical and molecular terms. In an effort to describe the phases in more detail, we used a large scale transcriptomic approach in the larvae of malt fly, *Chymomyza costata*, for which the environmental regulation of diapause development was established in our previous work (Koštál et al., 2000, J. Insect Physiol. 46, 417-428).

Design of experiment

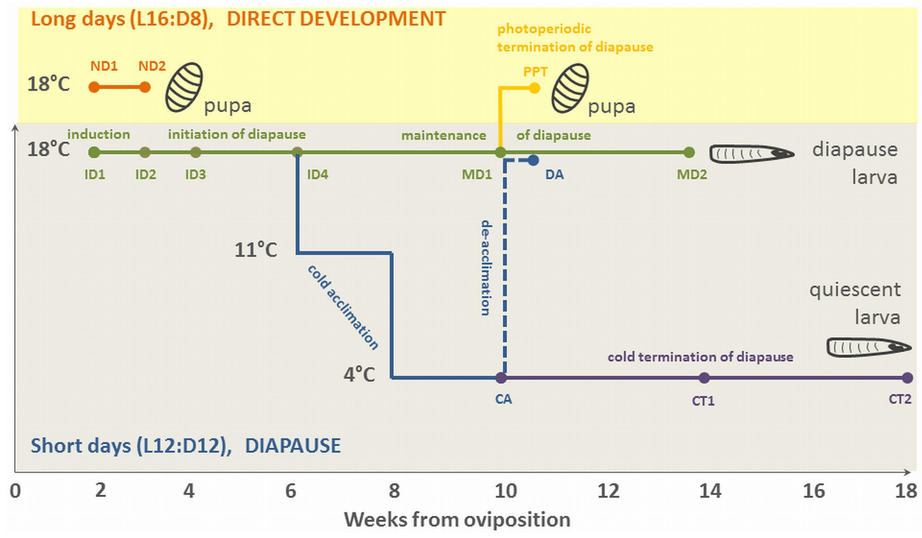

Fig. 1: Sampling scheme.

ND non-diapause

diapause:
ID initiation
MD maintenance
CA cold-acclimation
DA de-acclimation
CT cold termination
PPT photoperiodic termination

Experiment

Materials and Methods

The flies *Chymomyza costata* (Diptera: Drosophilidae) were cultured at constant temperature of 18°C on an artificial diet. Developmental destiny of larvae was programmed using two different photoperiodic regimes: a long day regime (LD, 16 hour light: 8 hour dark) at which all larvae continue direct development (*i.e.* pupariate, pupate and metamorphose to adults), and a short day regime (SD, 12 hour light: 12 hour dark) that induces larval diapause in all individuals. The larvae maintain their diapause under SD/18°C conditions for several months (until death). Diapause can be terminated either by cold (natural, horotelic process), which requires 2-3 months at 4°C, or by long photoperiod (un-natural, tachytelic process), which requires approximately two weeks under LD. After the horotelic termination, the larvae remain locked in developmental arrest at low temperatures (post-diapause quiescence) and can resume their development only upon exposure to high temperatures.

Total RNA was isolated from larval samples (see Fig. 1), converted to cDNA and subjected to microarray analysis using our own custom DNA microchips containing 1 047 oligonucleotide probes for candidate genes. Candidate genes were selected to cover a broad range of processes likely involved in the regulation of developmental arrest, metabolic suppression, cell division cycle, hormonal signaling, cold tolerance, metabolism of cryoprotectants, long-term maintenance of homeostasis including ionic balance, protection against aging, apoptosis and oxidative damage.

Here we present a preliminary analysis of principal components (PCA, Canoco, Biometris, Wageningen, the Netherlands) in transcriptomic patterns associated with individual phases of diapause development. Just a selected dataset concerning the phases of cold-acclimation and de-acclimation is analyzed in more detail in this poster in order to indicate the potential of the method for identification of candidate genes that might be later subjected to functional analysis.

Results and Discussion

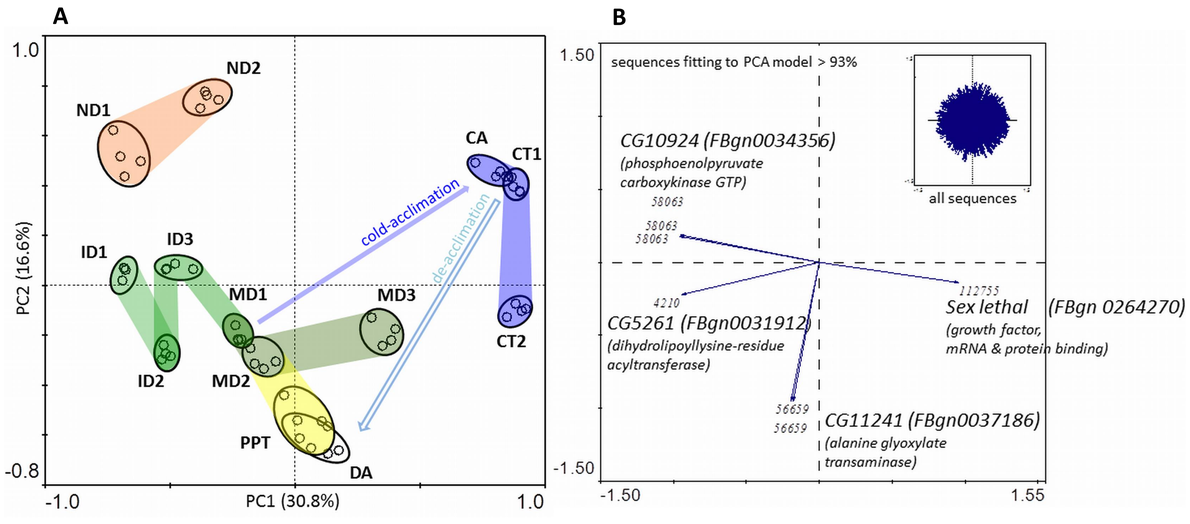

Fig. 2: Global transcriptomic patterns evolving under alternative developmental pathways including and not including diapause stage.

PCA of complex transcriptomic dataset nicely clustered the sample replications while clearly separating the treatments (various phases of diapause development, Fig. 2A). This result supports the view of diapause as dynamic process in which the insect continuously changes although it is developmentally arrested. The young and old 3rd instar non-diapause larvae (ND1 and ND2, respectively) are very clearly separated from young larvae that induce (ID1) or initiate (ID2, ID3) their diapause. This result was expected and it was well described in numerous earlier papers. More interestingly, the samples taken during diapause maintenance (MD1 - MD3) under constant SD/18°C conditions suggest that the transcriptomic composition develops over time. Larvae of *C. costata* are not able to terminate their diapause spontaneously under constant SD/18°C conditions. They require specific terminating stimuli: either exposure to low temperatures for long time (2-3 months) or exposure to long days for just 2-3 weeks. The larvae acquire very high level of freeze-tolerance (cold-acclimate) upon the decrease of ambient temperature to +4°C (CA). At this stage, however, their diapause is not yet terminated. They still maintain sensitivity to photoperiodic signal and upon return to permissive temperatures they maintain diapause under SD conditions but undergo de-acclimation (partially lose freeze-tolerance, DA). Exposure to cold leads to diapause termination when the larvae are exposed to +4°C for a long time period of 2-3 months. Termination is nicely supported by static transcriptomic pattern in samples CA and CT1 (no termination) but clearly separated patterns in samples CT1 and CT2 (termination). The transcriptomic pattern changes also with the photoperiodic termination (PPT) and overlaps with the pattern observed in DA larvae. Interestingly, PPT and DA patterns are clearly distant from ND pattern, which suggests that individuals with different ontogenetic history (diapause vs. non-diapause) are not equal. The PCA of global dataset returns some descriptors of transcriptomic changes (see eigenvectors of four sequences which fit the PCA model best, Fig. 2B, C). The global dataset, however, is too complex to identify major driving forces behind individual phases of diapause development.

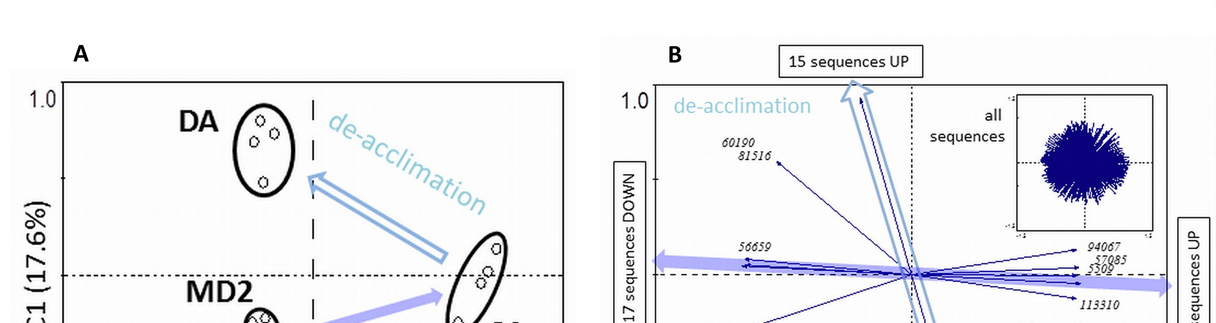

Fig. 3: Transcriptomic responses to cold-aclimation (CA) and de-acclimation (DA).

We will identify candidate genes for individual phases by performing PCA analyses using logical subsets of global dataset as shown on the example of CA and DA (Fig. 3A). The changes of temperature exerted dramatic influence on transcriptome. We detected 58 or 15 sequences that are strongly up-regulated or down-regulated, respectively, during cold acclimation. Perhaps not surprisingly, there are many Heat shock protein coding genes among the up-regulated sequences. Bolstered synthesis of cryoprotectants (proline and trehalose) at low temperatures is manifested by up-regulation of several sequences coding for relevant metabolic locuses or for trehalose transporter. Hormonal signaling might be affected by temperature as suggested by strong responses of genes coding for factors like Kruppel, JH binding protein, Broad, Leucokinin, or Insuline-like peptide 8 (Fig. 4A).

Most genes responding to CA did respond also to DA but in a reciprocal manner (compare CA and DA columns in a heat map on Fig. 4A). There was a small subset of genes in which the response to DA dominated and was not reflected by any reciprocal response to CA (Figs. 3B, 4B). These results suggest that transcriptomic responses to CA and DA are not simple mirror images but also contain some specific elements.

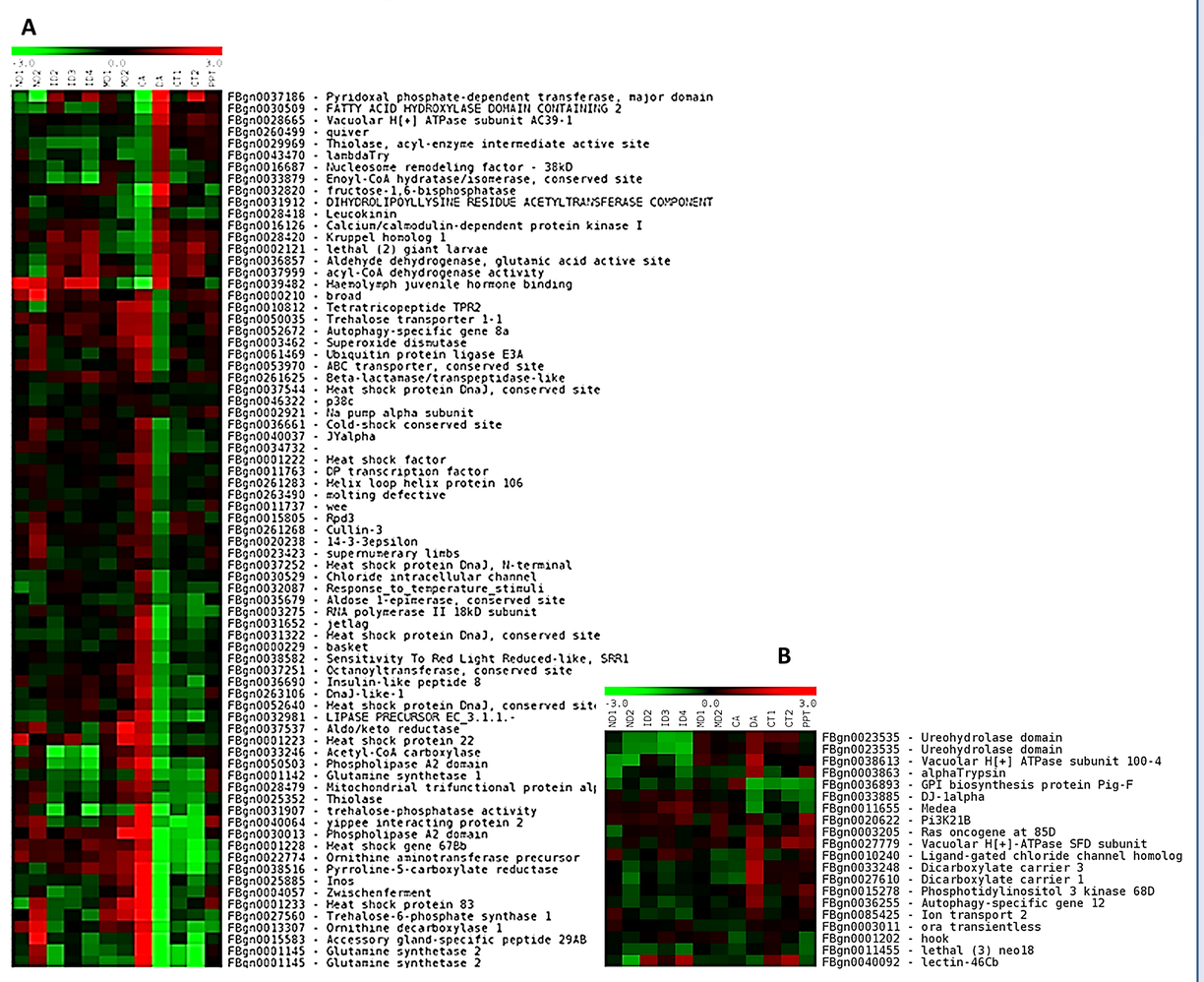

Fig. 4: Candidate gene-markers for cold-acclimation (CA) and de-acclimation (DA).

Conclusions

Good separation of different phases of diapause development in larvae of *C. costata* was achieved based on analysis of transcriptomic patterns using custom microarrays composed of 1 047 *C. costata*-specific DNA probes.

We continue in the **identification of candidate genes** that will serve as unique markers for different phases of diapause development and will be later subjected to functional analysis in order to recognize their roles in regulation of diapause or in cold-acclimation.

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