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Scientific report for the European Science Foundation programme 'Thermal adaptation in ectotherms: Linking life history, physiology, behaviour and genetics'

Applicant

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Project title

Effects of long term drought stress on cold tolerance and life history patterns in *Folsomia candida*

Hosts

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Background

Several studies indicate that many of the adaptations in ectothermic animals promoting cold tolerance were originally developed to tolerate desiccation stress (Block, 1996; Ring and Danks, 1994, 1998). In the true soil living Collembola species, *Folsomia candida*, drought acclimation at 98.2% relative humidity (RH) for 192 hours improves cold shock tolerance, at temperatures between -5 and 5°C for 48 hours, compared to cold tolerance in non-acclimated *F. candida* (Bayley et al., 2001; Holmstrup et al., 2002). This implies that at least some of the mechanisms behind adaptation to drought and low temperatures in *F. candida* are shared. These adaptations rely on desaturation of phospholipid fatty acids (Holmstrup et al., 2002) and accumulation of sugars and polyols (Bayley and Holmstrup 1999; Sjursen et al., 2001). *F. candida* and other euedaphic Collembola overcome desiccation by absorbing soil pore water vapour driven by hyperosmotic body fluids created from accumulation of glucose and myoinositol (Bayley and Holmstrup, 1999; Holmstrup et al., 2001). This accumulation results in an increase in internal osmolality from about 300 to 1250 mOsm/kg. Such metabolic perturbation in response to drought would be possible to detect on a transcriptomic level by studying the expression of genes coding for enzymes important in the metabolic pathways.

Adult *F. candida* moult continually throughout their lifetime with reproductive instars (1.5 days) followed by non-reproductive instars (8.5 days) (Fountain and Hopkin, 2005). Accomplishment of the moulting process is absolutely dependant on accumulation of exuvial fluid between the old and new cuticle (Krishnan, 1969). Moulting and reproduction ceases in *F. candida* when experiencing mild drought stress between 99.4% RH and 98.8% RH and is re-established when rehydrated after 5 weeks of drought (D. Waagner, unpublished studies). Thus the access to free water may be highly critical to *F. candida* to retain their normal life history patterns. Several enzymes are involved in degradation of the old cuticle and in the production of a new. Activity of these enzymes can be traced be analysing the expression of the genes coding for these enzymes. Thus the transcriptomic responses to long-term mild and strong drought and following rehydration may elucidate some of the crucial steps in *F. candida* reproduction.

Utilising the access to the library of more than 5000 cDNA sequences (www.collembase.org, Timmermans et al,. 2007) of *F. candida* perturbation of the metabolic pathways and moulting

processes can be studied on the transcriptomic level. The expression of candidate genes encoding for the enzymes of interest will be analysed by quantitative polymerase chain reaction (Q-PCR).

Purpose of the visit

In this project, comparative studies on the transcriptomic responses in drought exposed *F. candida* will be conducted to:

1) Evaluate the importance of the chosen candidate genes in relation to adaptation to low temperatures and mild and strong desiccation stress.
2) Associate transcriptional responses to desiccation and rehydration with recovery of moulting and reproduction.

The candidate genes in question could be coding for heat shock proteins (e.g.Hsp70), enzymes in relation to moult and egg production (e.g. chitanases and vitellogenin), and enzymes related to metabolic pathways (e.g. glucose-6-phosphate isomerase, trehalase).

Main results

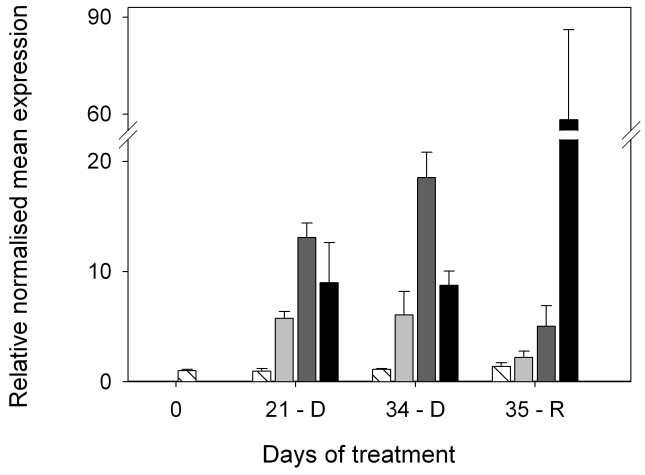
Drought experiments were conducted in Denmark previous to the visit. Samples were brought to VU, where isolation of mRNA, reverse transcription (to cDNA) and real time quantitative polymerase chain reaction (Q-PCR) was performed by me. In total, expressions of 7 target genes were analysed. Q-PCR data show that the transcription rate for all genes studied respond to drought and/or following rehydration in *F. candida*:

1) The transcription rates of heat shock proteins are differently affected by mild and severe drought. e.g in HSP70 (Fig. 1a).
2) The effect of drought and rehydration on moulting can be correlated with the transcription rate of a moulting related gene, Chitinase 1 (Fig. 1b).
3) The transcription rate of a glycolytic enzyme, glucose-6-phosphate isomerase, is increased in response to mild drought and re-established after rehydration, indicating increased isomerisation between glucose-6-phosphate and fructose, probably in the direction of glucose-6-phosphate (1c).

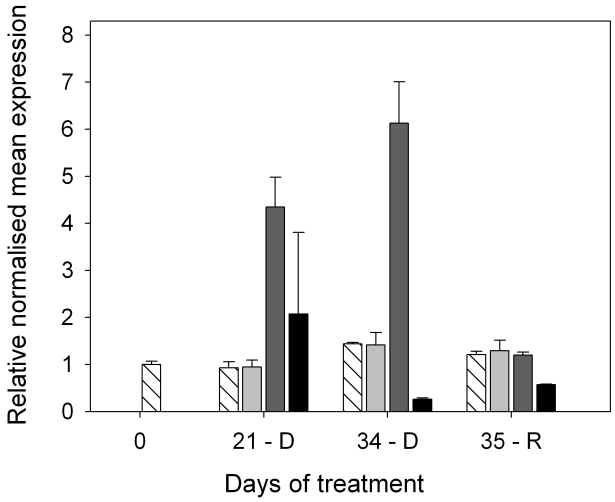
Figure 1a-c

Expression of a) Heat shock protein 70, b) Chitinase 1 and c) Glucose-6-phosphate in all treatments at all times. Gene expression is relative to the mean of control at day 0 and normalised to the expression of the reference gene (Succinate Dehydrogenase Complex Subunit A, SDHA). Crossed bars: 100% RH, petri dish, Light grey bars: 99.8% RH, Dark grey bars: 98.2% RH, Black bars: 96.1% RH.

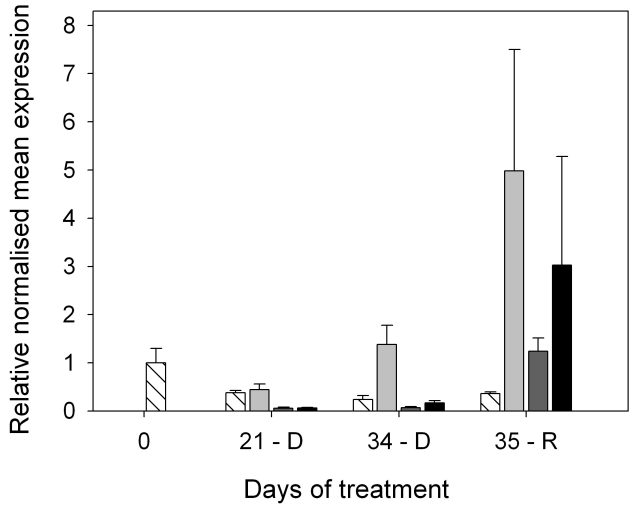
a)



c)



b)



Publication and further collaboration

Future work includes data analysis and writing for publishing in a peer reviewed journal. This work will mainly be performed by the applicant in Denmark.

A future visit at VU within the following months is planned. This visit will improve the writing process plus provide opportunity for planning of future work and collaborations.

Comments

This grant gave me the opportunity to gain further experience with Q-PCR and work focused on the described project. Furthermore, this exchange stay strengthened the cooperation with the host institution. I am therefore very grateful to ESF (ThermAdapt) for the financial support.

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