Final report of project:

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Project title: "Linking variation in heat shock resistance at the phenotypic level to gene- and protein-expression in Hsp70 in collembolans"

Host: Professor Jacintha Ellers, Department of Animal Ecology, Institute of Ecological Science, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

Purpose:

The purposes of my 12-week visit to the Vrije Universiteit, Amsterdam were to study the link between heat shock response at the phenotypic and molecular levels in thermal adaptation to heat stress using a set of inbred isofemale lines of the collembolan species *Orchesella cincta*. Our three main goals were:
1. Establish the variation in heat resistance, heat shock protein (Hsp70) expression and Hsp70 gene expression. The inducible Hsp70 will be the target of both the protein and gene level expression because Hsp70 has been shown highly important for heat resistance.
2. Establish the link between these different levels, (heat resistance, Hsp70 protein and gene expression) to investigate genetic variation at the population level and correlate the differences in heat shock resistance between lines with gene and protein expression.
3. Look at the effect of inbreeding on both Hsp70 expression and heat resistance in the inbred lines compared to outbred populations, which both originate from the same starting population. Inbreeding is known to affect both heat shock protein expression and heat resistance in fruit flies (Kristensen et al., 2002), and is expected to become more and more important with the increasing fragmentation of natural habitats. It is therefore crucial that we can quantify this effect on the thermal response.

Description of the work carried out during the visit:

We were able to follow our schedule and planned aims and were therefore able to look at the following subjects (although some results are still waiting):

1. Establish the variation in heat resistance, heat shock protein (Hsp70) expression and Hsp70 gene expression: We have been able to obtain some very nice results on the genetic variation in heat resistance (fig. 1). In concordance with earlier studies (Bahrndorff et al., 2006) we observed ample genetic variation between isofemale lines, which is promising for assessing the amount of variation on Hsp70 expression at RNA and protein level.
2. Correlate the protein and gene expression with heat shock resistance between isofemale lines: results are shortly to be expected.
3. Look at the effect of inbreeding on both Hsp70 expression and heat resistance in the inbred lines: There seems to be an effect of inbreeding on heat shock resistance. However, due to the number of outbred populations used, additional studies would add useful information on this topic.
4. Look at the effect of heat hardening on both Hsp70 expression and heat resistance in massbred population: We were able to include some work on heat hardening (fig. 2), which is of great

importance in understanding heat shock resistance and Hsp70 expression. The results show that heat shock resistance is increased significantly after hardening, and also that the effect of heat hardening persists for a long time compared to species such as Drosophila.

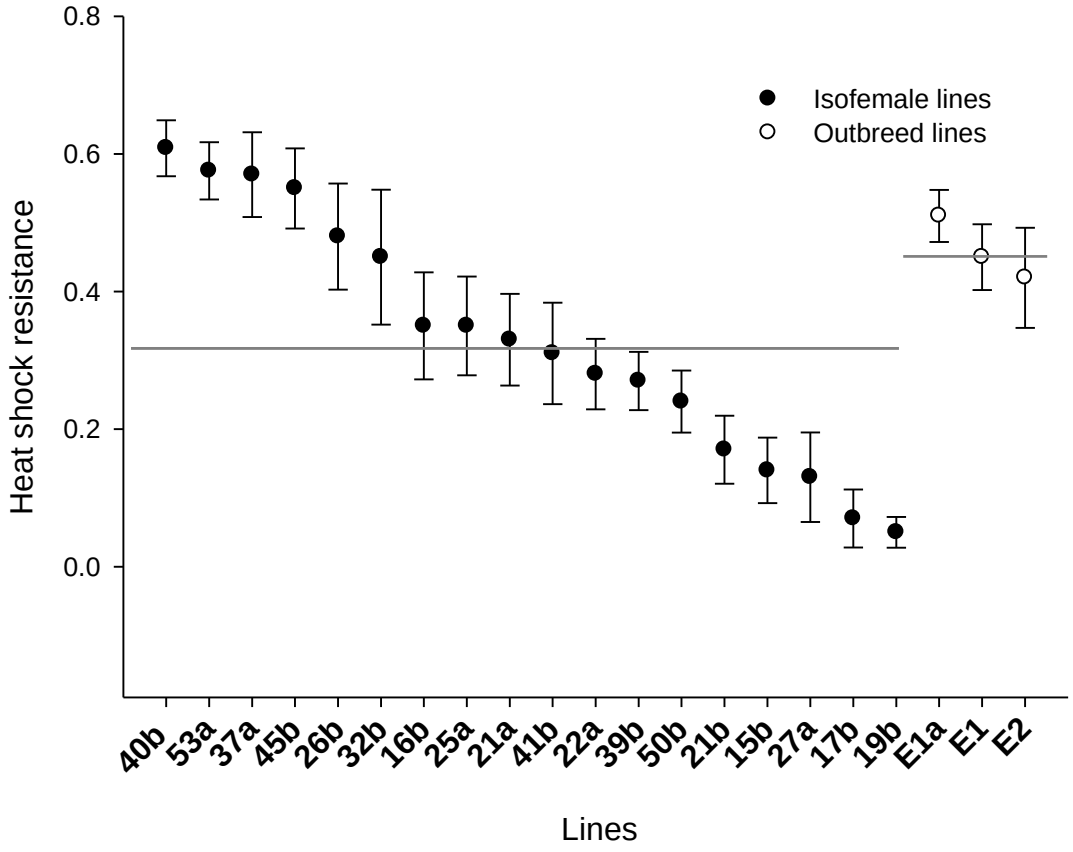

Figure 1. Heat shock resistance (measured as survival proportion) after 22 hours of recovery (mean ± SE), sorted by mean number of survivors in inbred and control lines. Mean heat resistance within breeding regimes is indicated by the solid grey horizontal line.

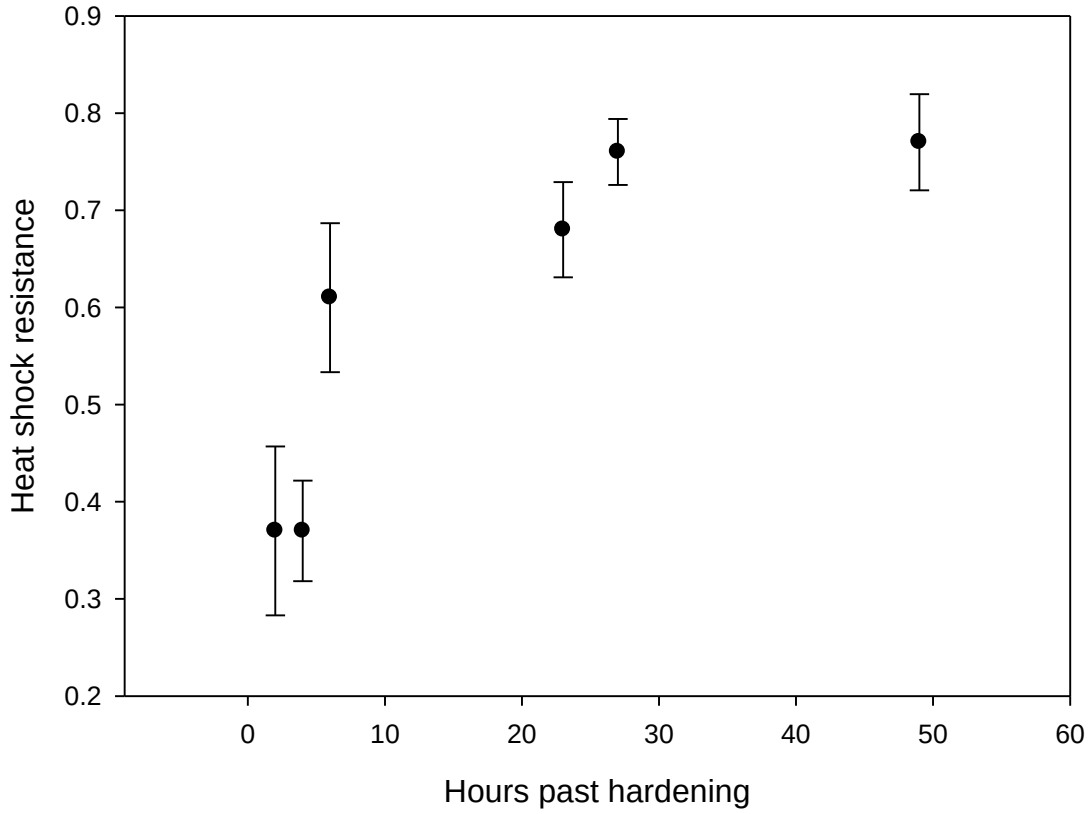

Figure 2. Heat shock resistance (measured as survival proportion) after 1 hour at 37.4 Cº (mean ± SE). Survival was estimated at different times elapsed between heat hardening and exposure to heat shock. Survival without prior heat hardening was low (0.30).

Future collaboration with host institution: An application has been submitted for joint collaboration on drought resistance in Collembola. Both Danish (Carlsberg and National Danish Science Foundation) and Dutch grants (Rubicon) have been submitted.

Projected publications to result from the grant: (Two articles are to be expected)

1: Title: "Genetic variation for stress resistance and Hsp70 expression in inbreed isofemale lines of the springtail *Orchesella cincta*."

2: Title: "Induced heat stress resistance and of gene- and protein-expression in Hsp70 in springtails, *Orchesella cincta*."

Comments: This grant has been very useful for both parts of the project and strengthened the cooperation between the institutions. We are therefore very grateful to ESF for the grant.