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**Direct and trans-generational effects of heat shock on life-history traits
in a soil arthropod**

Host

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Purpose of the visit

Studies of parental effects in an ecological context provide important insights into the evolution of adaptation under variable environmental condition. Temperature is a key environmental factor for ectotherms (Hoffmann et al. 2003), and parental temperature has been found to have diverse phenotypic effects on offspring traits (Crill et al. 1996, Steigenga & Fischer 2007).

Trans-generational effects have been found to be particularly relevant under environmental stress (Ho & Burggren 2010), however little is known about how parental temperature stress affects offspring fitness and if trans-generational effects of temperature last when offspring gets older.

An ESF grant was awarded to me in 2009 to explore the effects of thermal stress on male fitness in the soil arthropod *Orchesella cincta*. I showed that temperature stress decreased reproductive performance of male *O. cincta* by inducing a delay in first reproduction and reducing attractiveness of males to females (Zizzari & Ellers 2011).

These results prompted me to investigate the effects of heat stress on female fitness and whether mothers exposed to heat shock have a long-term effect on offspring thermal sensitivity.

My main goals were:

1. Quantify detrimental effects of heat shock on female fitness and survival
2. Investigate whether heat shock experienced by females has an effect on progeny thermal sensitivity at different life stages
3. Test whether the heat shock response varies according to the life stages

Description of the work carried out during the visit

I studied the effect of maternal heat shock on offspring fitness in a laboratory population of *O. cincta*. I have chosen this springtail to address my questions because it occurs in habitats with highly fluctuating temperatures in soil ecosystems and therefore needs to cope with thermal stress. The small body size, the short generation time and the excellent knowledge on the reproductive biology of *O. cincta* (Ernsting & Isaaks 2002; Gols et al. 2004; Zizzari et al. 2009) make this species highly suitable for investigating trans-generational effects of temperature. Furthermore, several aspects of the thermal responses in this species have already been studied (Driessen et al. 2007; Ellers et al. 2008; Bahrndorff et al. 2009, 2010; Zizzari & Ellers 2011).

Temperature treatments

I used an *O. cincta* strain that has been kept at the VU University for 3 generations under outbred conditions. The animals were kept under standard conditions at 20°C, 12:12 L:D, in pots with plaster of Paris to ensure sufficiently high humidity, and with small branches covered with algae for food.

The animals were exposed to two treatments:

1. Heat shock: exposure to 36.5°C for 1h
2. Control: handled like the other treatment, but not exposed to heat (20°C)

The heat shock temperature was chosen to induce very little mortality (based on Zizzari & Ellers 2011) to avoid selection effects. Before and after the treatments the animals were kept individually in small vials at 20°C. To initiate the experiments, *O. cincta* eggs (F_0) were collected from several hundred adults, and reared to maturity at 20°C.

Effects of thermal stress on female life history traits

I sexed 160 females. The individuals were exposed to the two treatments described above, with 8 replicate vials with 10 individuals each for each treatment. I thus obtained two maternal generations, one exposed to brief heat shock and the other to 20°C.

Two days after exposure, the treated females (F_0) were allowed to reproduce with untreated males. Eggs (F_1) collected from each of the maternal temperature group were placed in plastic vials at 20°C.

Survival of females (F_0) was assayed 72 h after exposure. To assess female fitness, clutch size and hatching success were recorded.

After hatching, a random subset of juveniles (F_1) from each maternal generation was placed individually in vials for the subsequent trans-generational experiment.

Trans-generational effects of thermal stress

To evaluate the effects of thermal stress on progeny generation, heat stress resistance was monitored at three time points: during the egg stage, the juvenile stage and as adults.

To determine whether maternal effects of heat shock decay as an individual gets older I reared 2/3 of the total offspring (F_1) to maturity, while a subset of clutches (F_1) was used to measure thermal resistance of eggs.

I exposed each of the three F_1 groups of both maternal generations to 36.5°C and assayed their survival. For the treatments I used eggs one day old, juveniles of 20 days and adults of 45 days. Survival of all groups was assessed 72 hours after the treatments.

Description of the main results obtained

Survival of females (F_0) was assayed and I found that 18% of the animals exposed to 36.5 °C died. The proportion of surviving animals was significantly higher when they were exposed to 20°C (χ^2 -test, $P < 0.05$).

Female fitness was assessed by measuring clutch size and hatching success.

One-way ANOVA analysis showed that the clutch size differed significantly between the treatments, with a higher proportion of eggs (χ^2 -test, $P < 0.05$) in the control treatment.

However, I did not find a significant reduction in hatching success in the heat treatment compared to the control (χ^2 -test, $P > 0.05$).

I tested whether heat shock experienced by females has an effect on offspring thermal sensitivity. The experiment was designed to compare the survival after heat shock of the progeny of the untreated maternal generation (20°C) and of the maternal generation exposed to heat stress (36.5 °C).

Eggs hatching success following exposure to heat shock did not show significant differences between the two F_1 groups (χ^2 -test, $P > 0.05$). Mortality of juveniles resulted to be very low, regardless of the thermal environment experienced by the mothers (χ^2 -test, $P > 0.05$).

However, mothers affected thermal sensitivity of the progeny at adult life stage. Survival of adults was much higher when the maternal generation was exposed to heat stress (χ^2 -test, $P < 0.05$).

I then recorded life stage-related differences in thermal tolerance. Among the stages tested, juveniles showed maximum tolerance, whereas eggs showed minimum tolerance (χ^2 -test, $P < 0.05$).

My results indicate that even a brief heat shock can affect female fitness and that heat shock experienced by females indeed has an effect on progeny thermal sensitivity in the collembolan *O. cincta*. However, not all stages were affected in the same way by the maternal thermal stress.

The survival rate following heat stress, resulted significantly higher in the progeny of mothers exposed to heat shock than in the progeny of untreated mothers, but only in the adult stage (χ^2 -test, $P < 0.05$), whereas no significant differences were found in the egg and juvenile stages (χ^2 -test, $P > 0.05$).

I thus showed that maternal thermal environment may persist through to the adult stage of the progeny.

Also, the heat shock response is enhanced in juvenile compared with adult springtails, suggesting that temperature thresholds vary among *O. cincta* life stages.

Future collaboration with host institution and projected publications

I applied for a postdoctoral fellowship to conduct research at the Department of Animal Ecology, VU University of Amsterdam.

The data obtained from the ESF project are expected to be published at least in one paper.

Comments

This grant gave me the opportunity to strengthen the cooperation with the host institution. I am therefore very grateful to ESF.

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