UNIVERSITA' DEGLI STUDI DI SIENA
DIPARTIMENTO DI BIOLOGIA EVOLUTIVA

Via Aldo Moro 2 – 53100 Siena

Dr Z. Valentina Zizzari
E-Mail: valentina.zizzari@falw.nl.vu
zizzari@unisi.it

Direct and indirect effects of heat shock on male reproductive fitness in the Collembola *Orchesella cincta*

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 of the visit:

Temperature is one of the main ecological determinants of performance in ectotherms. Exposure to thermal stress can not only cause severe mortality, but also significantly impair the fitness of surviving individuals. Reproductive traits are more sensitive to thermal stress than are other life history traits, therefore they are relevant to estimate the fitness effects of thermal stress (Jørgensen et al. 2006).
I studied the effect of heat shock on male fertility and mating success in the collembolan *Orchesella cincta*. I have chosen this organism because it occurs in habitats with highly fluctuating temperatures in soil ecosystems and therefore needs to cope with thermal stress. Several aspects of the thermal responses in this species (Driessen et al. 2007; Ellers et al. 2008; Bahrndorff et al. 2009) and its reproductive biology (Zizzari et al. 2009, Gols et al. 2004; Ernsting & Isaaks 2002) have been studied before. *O. cincta* reproduces by dissociated sperm transfer, thus males deposit sperm droplets (spermatophores) on the soil without the presence of females. Therefore male reproductive fitness is easily estimated in this species by counting the number of spermatophores. Also, females are known to perform mate choice, even without the male's presence, probably using olfactory cues associated with the spermatophores (Zizzari et al. 2009).
My main goals were:
1. Quantify detrimental effects of heat stress on male reproductive traits measuring overall reproductive ability.

2. Test whether induced thermotolerance can prevent detrimental effects on reproduction.
3. Investigate the effect of heat shock on male mating success by measuring male attractiveness to females.
4. Estimate the fitness consequence of male mating success.

Description of the work carried out during the visit:

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 four treatments:
1. Heat shock: Exposure to 37.2°C for 1h.
2. Heat hardening: Exposure to a mild heat stress of 35.2°C for 1h.
3. Heat hardening + heat shock: Exposure to 35.2°C for 1h, followed 15h later by 37.2°C for 1h.
4. Control: Handled like the other treatments, but not exposed to heat (20°C).
Each treatment consisted of 55 animals. 5 individuals per vial and 11 vials per treatment were tested.
The heat shock temperatures were chosen to induce no mortality (based on Bahrndorff et al. 2009). Before and after the treatment the animals were kept individually in small vials at 20°C. In the treatments I used juveniles 33 days old because, although Hsps are ecologically relevant for all life stages, juveniles are especially dependent on Hsps for survival (Sørensen et al. 2003).

Effect of heat stress on survival and reproduction

Springtails were assayed for thermal resistance after exposure to the temperature treatments. After exposure individuals were allowed to recover for 24 h before mortality was assayed. I sexed the survivals and measured male fertility by daily counting the number of spermatophores produced during the first reproductive instar. Post-stress time to gain the fertility was also recorded.

Female choice trials

I used experimental arenas consisting of plastic vials with a bottom of plaster of Paris. Each arena contained four spermatophore patches. Every patch consisted of two spermatophores from a male of one of the four treatment groups. A virgin, receptive, female was introduced to the arena and allowed to take up a spermatophore (cf. Zizzari et al. 2009). Female behaviour was observed throughout the entire trial through a stereomicroscope. A trial ended once a female takes up a spermatophore or after 15 minutes. Vials were checked for eggs each subsequent day until egg laying stopped. The number of the offspring was also recorded.

Description of the main results obtained:

Survival to heat stress was checked and I found that 20% of the animals exposed to 37.2°C (heat shock) and 25% exposed to 35.2°C followed by 37.2°C (heat hardening + heat shock) died. The proportion of surviving animals was significantly higher when they were exposed to 35.2°C and 20°C (χ^2-test, $P<0.05$). The control (20°C) did not differ significantly from the heat hardening (35.2°C).

Because the sex of juvenile *O. cincta* cannot be determined visually, the animals were sexed after having reached maturity that was after the heat treatments.

- Out of 46 survival exposed to 37.2°C 26 were males.
- Out of 54 survival exposed to 35.2°C 27 were males.
- Out of 44 survival exposed to 35.2°C +37.2°C 27 were males.
- Out of 54 survival exposed to 20°C (control) 26 were males.

The spermatophore production of the males was recorded for the first reproductive instar after the treatments. If males had not produced spermatophores 15 days after exposure they were regarded as permanently sterile males. I found only one male belonging to the heat shock treatment permanently sterile. The proportion of males that remained sterile did not differ significantly among the heat treatments (χ^2-test, $P>0.05$).

The time to reach first reproduction after heat stress was analyzed by a non-parametric Kruskal–Wallis test. The average age at maturity of males varied significantly between the heat treatments. The heat shock treatment (37.2°C) had a significant longer effect on time to gain fertility ($P<0.05$). However, the pretreatment (35.2°C+37.2°C) did not reduce significantly the negative effect of the heat shock ($P>0.05$).

The pattern of spermatophore production of males in their first reproductive instar revealed high variability among males. However One-way ANOVA showed that the average spermatophore production did not differ significantly between the heat treatments ($P>0.05$). Also, surviving males that were heat stressed without pretreatment (37.2°C) were not significantly less likely to produce spermatophores than were males in the other heat treatments ($P>0.05$).

In conclusion, I did find direct effects of heat shock on male reproductive functions in *O. cincta.* These effects were only related to the time to gain fertility and not to spermatophore production.

However, heat shock may also affect male reproductive success indirectly, by reducing sexual attractiveness of males to females (Fasolo & Krebs 2004). I therefore measured female mate choice among spermatophores of males subjected to the different degree of thermal stress.

In the female choice trials 38 tested females made a choice and took up a spermatophore; 16 took up a spermatophore from the control patch, which is significantly more than could be expected by chance (χ^2-test, $P<0.05$).

The 38 choosy females were then checked for egg production. Out of the females that did not produce eggs, 5 chose spermatophores of males exposed to 37.2°C, which is significantly more than could be expected by chance (χ^2-test, $P<0.05$).

The average number of offspring produced from spermatophores of males stressed at different levels of heat did not differ significantly (One-way ANOVA, $P>0.05$). For the males stressed at 37.2°C, 35.2°C and 35.2°C+37.2°C, the offspring production remained at control levels.

In conclusion, female mate choice is affected by male thermal history because females seem prefer males that have not been exposed to heat shock. Offspring production of males that were exposed to heat shock is compromised because a higher percentage of their spermatophores caused reproductive failure in females.

Future collaboration with host institution:

A Rubicon fellowship has been awarded to me to conduct research at the Department of Animal Ecology, VU University, with Jacintha Ellers.

Projected publications:

Title:" Direct and indirect effects of heat shock on male reproductive fitness in the Collembola *Orchesella cincta*". This publication is in preparation.

Comments:

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

References

Bahrndorff, S., Marien, J., Loeschcke, V. & Ellers, J. 2009. Dynamics of heat-induced thermal stress resistance and Hsp70 expression in the springtail, *Orchesella cincta*. *Functional Ecology*, 23, 233-239.

Driessen, G., Ellers, J. & Van Straalen, N. M. 2007. Variation, selection and heritability of thermal reaction norms for juvenile growth in *Orchesella cincta* (Collembola: Entomobryidae). *European Journal of Entomology*, 104, 39-46.

Ellers, J., Marien, J., Driessen, G. & Van Straalen, N. M. 2008. Temperature-induced gene expression associated with different thermal reaction norms for growth rate. *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution*, 310B, 137-147.

Ernsting, G. & Isaaks, J. A. 2002. Gamete production and sexual size dimorphism in an insect (*Orchesella cincta*) with indeterminate growth. *Ecological Entomology*, 27, 145-151.

Fasolo, A. G. & Krebs, R. A. 2004. A comparison of behavioural change in *Drosophila* during exposure to thermal stress. *Biological Journal of the Linnean Society*, 83, 197-205.

Gols, R., Ernsting, G. & van Straalen, N. M. 2004. Paternity analysis in a hexapod (*Orchesella cincta*; Collembola) with indirect sperm transfer. *Journal of Insect Behavior*, 17, 317-328.

Jørgensen, K. T., Sørensen, J. G. & Bundgaard, J. 2006. Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. *Journal of Thermal Biology*, 31, 280-286.

Sørensen, J. G., Kristensen, T. N. & Loeschcke, V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, 6, 1025-1037.

Zizzari Z.V., Braakhuis A., van Straalen N.M. & Ellers J. 2009. Female preference and fitness benefits of mate choice in a species with dissociated sperm transfer. Animal Behaviour, 78, 1261-1267.