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Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod

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ABSTRACT

Ambient temperature is a key environmental factor influencing a variety of aspects of the ecology and evolution of ectotherms. Reproductive traits have been suggested to be more sensitive to thermal stress than other life history traits. This study investigated the direct and indirect effects of heat shock on male reproductive success in the widespread springtail *Orchesella cincta*. Male springtails were exposed to four temperature treatments: heat hardening (35.2 °C for 1 h), heat shock (37.2 °C for 1 h), heat hardening + heat shock (35.2 °C for 1 h, followed 15 h later by 37.2 °C for 1 h), and control (20 °C). The heat shock gene *Hsp70* showed high expression in all the heat treatments, indicating that the treatments indeed induced thermal stress. Significant mortality was only found in the treatment with heat shock, both with and without heat hardening. A direct effect of heat treatment was found on time to first reproduction, which was significantly longer after heat shock (with or without heat hardening) than in the control treatment. There was no difference among treatments in the number of spermatophores produced in the first reproductive instar. Heat treatment also had indirect effects on male reproductive success. Females chose significantly more spermatophores from control males than from males that received heat shock, heat hardening or both. A high percentage of spermatophores produced by heat shocked males caused reproductive failure in females, but no significant differences among treatments were found.

Our results suggest that not all traits were equally affected by the heat stress. Heat hardening did not protect reproductive traits against the negative effects of heat shock. The indirect effects of heat shock on reproduction may be equally important as the direct effects.

1. Introduction

With global climate change becoming increasingly evident, understanding the physiological responses of organisms to these changes is a priority (Mydlarz et al., 2010). More frequently occurring heat waves and prolonged drought periods are some of the physical and biological changes associated with global warming. It is difficult to predict how these aspects can affect the ecosystem functioning and species diversity because species differ in their response to changing climatic conditions (Berg et al., 2010). Even short-lived extreme events can have long-lasting effects on aspects of an organism's fitness, resulting in unexpected shifts in community composition (Jentsch et al., 2007). To predict how environmental changes affect biological systems we need an understanding of how such changes will impact not just survival of individuals but also their reproductive success.

Ectotherms are especially vulnerable to climate warming because their basic physiological functions are strongly influenced by environmental temperature (Deutsch et al., 2008). There are several ways in which ectotherms can moderate the detrimental effects of exposure to high temperatures. First, heat shock elicits the production of heat shock protein. It is well-established that heat shock proteins play a major role in heat resistance by preventing damage due to denaturated protein aggregations in heat-exposed organisms (Feder and Hofmann, 1999). Furthermore, previous exposure to non-lethal high temperatures improves heat shock survival for a prolonged period of time, a response called heat hardening (Krebs and Loeschcke, 1994a,b; Dahlgaard et al., 1998; Bahrndorff et al., 2009). Heat hardening is also associated with the induction of heat shock proteins, especially Hsp70 (Bettencourt et al., 2008; Bahrndorff et al., 2009). On the other hand, the production of heat shock proteins and the associated heat hardening is costly and causes a reduced fecundity (Krebs and Loeschcke, 1994a) and a decrease of growth rate and cell division (Feder et al., 1992; Krebs and Feder, 1997).

Non-lethal effects of heat stress have received relatively little attention. These are often measured as alteration in locomotory performance or metabolic rate (Roberts et al., 2003; Cano and Nicieza, 2006; Dahlhoff et al., 2008). However, reproductive traits are thought to be more sensitive to thermal stress than other traits;

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therefore, they should be included when estimating the fitness effects of thermal stress (Jørgensen et al., 2006). Several studies have investigated the negative effects of heat shock on reproduction in both males and females of different insect species (Rinehart et al., 2000; Huang et al., 2007; Cui et al., 2008; Mironidis and Savopoulou-Soultani, 2010; Roux et al., 2010). In male *Drosophila*, the most studied model, heat stress often causes sterility or reduced fertility (Krebs and Loeschcke, 1994a,b; Sarup et al., 2004; David et al., 2005; Jørgensen et al., 2006).

In addition to the direct effects on male reproductive functions, heat shock may affect male reproductive success indirectly, by reducing sexual attractiveness of males to females (Fasolo and Krebs, 2004). Rohmer et al. (2004) and Krebs and Thompson (2005) showed that thermal stress in male *Drosophila* affects courtship behaviour by causing anatomical injuries. In *Drosophila mojavensis*, heat stress makes males less attractive to females because of changes in the composition of epicuticular hydrocarbons (Markow and Toolson, 1990), but studies for other species are lacking. Whether an organism protected against the lethal effects of an exposure is also protected against detrimental effects to its attractiveness still needs further investigation.

Here we study the direct and indirect effects of heat shock on male reproduction in the collembolan *Orchesella cincta*. *O. cincta* is a wingless arthropod that lives in the litter layer on the soil surface. It occurs in habitats with highly fluctuating temperatures (Liefting and Ellers, 2008) and therefore needs to cope with thermal stress. Several aspects of the thermal responses in this species have been studied before, including genetic variation in thermal reaction norms (Driessen et al., 2007; Ellers et al., 2008), geographic clines in thermal resistance (Bahrndorff et al., 2006) and the dynamics of heat hardening and associated gene expression (Bahrndorff et al., 2009). In addition, its reproductive biology is well-known (Ernsting and Isaaks, 2002; Gols et al., 2004; Zizzari et al., 2009). *O. cincta* reproduces by dissociated sperm transfer, in which males deposit sperm droplets (spermatophores) on the soil without the presence of females. Therefore male gametic output is easily estimated in this species by counting the number of spermatophores. Also, females have been found to choose among spermatophores (Zizzari et al., 2009), which allows an estimation of the indirect effects of heat shock on male reproduction.

In this study we address three main questions. First, how does heat-shock, with and without heat hardening, affect fecundity of males. We quantified potential detrimental effects of heat stress on male reproductive traits by measuring time to first reproduction and the number of spermatophores produced in the first reproductive instar. Also, we assessed levels of *Hsp70* expression to ensure that the heat shock elicited a response.

Second, is male mating success affected by thermal stress conditions experienced previously in life? Attractiveness of spermatophores from males which had been subjected to different heat shock treatments was measured employing female choice experiments. Third, we tested if heat shock affected the number of offspring produced per spermatophore. Although fecundity is normally associated with offspring production by female, it is documented that heat shock in male adults may affect their progeny due to a reduction in the rate of egg hatching (Silbermann and Tatar, 2000; Jørgensen et al., 2006). We therefore tested the fertility of the males exposed to the different heat treatments.

2. Materials and methods

2.1. Study species

O. cincta (Linnaeus, 1758) (Entomobryidae) is a surface-dwelling springtail species (adult size 3–4 mm), found in the litter layer in a broad range of habitats in the Holarctic (Timmermans et al., 2005; Liefting and Ellers, 2008). *O. cincta* is an ametabolous hexapod that grows indeterminately. Adults alternate reproductive and non reproductive periods (instars), separated by moults (Joosse et al., 1973). At 20 °C, reproductive instars generally last 5 days; non reproductive instars 4 days. Males deposit spermatophores even in the absence of females, but only during the reproductive instars. The female's receptive phase is limited to 48 h after the last moult (Joosse, 1981). After moulting into receptivity, a female takes up only one spermatophore to fertilize all her eggs and oviposition usually starts about 1 h after the spermatophores have been taken up (Gols et al., 2004).

All animals used in this study originated from a pine forest in The Netherlands (Roggebotzand [52°34.40N, 05°47.90E]) and were maintained as a stock population at 16 °C in a climate-controlled room (70% relative humidity, 12:12 h light:dark). Animals were kept in plastic vials with a moistened bottom of plaster of Paris, which kept humidity inside the vials at nearly 100%. Pine tree twigs covered with green algae (*Desmococcus* sp.) were provided for food and were regularly replenished.

2.2. Temperature treatments

For the heat shock experiments, eggs were isolated and kept at 20 °C (12:12 h light:dark) with excess food and checked daily for hatchlings. When juveniles were 30–33 days old they were transferred to glass vials containing slightly moistened foam at the bottom and moistened foam stoppers. Vials were put for 60 min in a water bath and exposed to one of four temperature treatments. We used juveniles because, although Hsps are ecologically relevant for all life stages, juveniles are especially dependent on Hsps for survival as a result of their high stress sensitivity and often low mobility (Sørensen et al., 2003).

The four temperature treatments were (1) heat hardening, which involved exposure to a mild heat stress of 35.2 °C for 1 h; (2) heat shock, which involved exposure to 37.2 °C for 1 h; (3) heat hardening + heat shock, which involved exposure to 35.2 °C for 1 h, followed 15 h later by 37.2 °C for 1 h; and (4) a control treatment in which the animals were not exposed to heat, but they were otherwise handled like the other treatments and kept at 20 °C. After the treatments, individuals were transferred back to control conditions. The heat hardening and the heat shock temperatures were chosen to induce low to intermediate levels of mortality based on a prior pilot experiment and previous work by Bahrndorff et al. (2009, 2010). Each treatment consisted of 55 animals comprising 5 individuals per vial and 11 vials per treatment. Before and after the treatment the animals were kept individually in small vials at 20 °C.

2.3. RNA extraction and quantitative RT-PCR analysis

To ensure that the heat shock treatment indeed elicited a heat shock response we measured expression levels of *Hsp70*. Five individuals per vial and two vials per treatment were used for mRNA analysis. After the treatment animals were allowed to recover for 1 h and then frozen in liquid nitrogen and stored at −80 °C. Animals were crushed and RNA was isolated using SV Total RNA isolation system (Promega). For cDNA synthesis 5 μL of total RNA (approximately 100 ng RNA μL^{-1}) was reverse transcribed using 200 U MML-V reverse transcriptase (Promega) and 0.5 μg oligo(d)T, according to the manufacturer's instructions. The cDNA samples were 1:3 diluted and 2 μL was used in 20 μL PCR reaction volumes containing forward and reverse Hsp70-RT primers (Bahrndorff et al., 2009) and Power SYBR Green PCR Master Mix (Applied Biosystems). Quantitative RT-PCR was performed in triplicate for each sample as described above. A mean normalized expression value (MNE) was calculated from the obtained Ct values