

How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)?

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Abstract

Because insects are ectotherms, their physiology, behaviour and fitness are influenced by the ambient temperature. Any changes in environmental temperatures may impact the fitness and life history traits of insects and, thus, affect population dynamics. Here, we experimentally tested the impact of heat shock on the fitness and life history traits of adults of the aphid parasitoid *Aphidius avenae* and on the later repercussions for their progeny. Our results show that short exposure (1 h) to an elevated temperature (36°C), which is frequently experienced by parasitoids during the summer, resulted in high mortality rates in a parasitoid population and strongly affected the fitness of survivors by drastically reducing reproductive output and triggering a sex-dependent effect on lifespan. Heat stress resulted in greater longevity in surviving females and in shorter longevity in surviving males in comparison with untreated individuals. Viability and the developmental rates of progeny were also affected in a sex-dependent manner. These results underline the ecological importance of the thermal stress response of parasitoid species, not only for survival, but also for maintaining reproductive activities.

Keywords: developmental rate, fecundity, heat stress, longevity, sex-specific effect, parasitic wasp

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Introduction

In insects, body temperature depends on the ambient temperature; and physiological functions, behaviour (e.g. flight, foraging, courtship, mating, oviposition) and fitness (e.g. developmental rate, lifespan, fecundity, gametogenesis) are directly affected by its fluctuations. As in all ectothermal

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organisms, insects show an optimal temperature preference to which their physiological functions are best adapted (Angilletta *et al.*, 2002; Chown & Terblanche, 2006). At temperatures higher than optimal, insects are stressed and activity costs are higher, inducing behavioural and physiological changes that often affect fitness (Angilletta *et al.*, 2002; Chown & Terblanche, 2006).

Global climatic change involves not only an increase in average temperatures, but also an increase in the intensity and frequency of extreme climatic events such as heat waves (Easterling *et al.*, 2000). Parasitoid insects represent a third trophic level, as they develop inside of their insect hosts that generally feed on plants. Parasitoids are keystone species that must deal with their own thermal stress, as well as that of their host. As such, it is to be expected that higher temperatures can have a severe impact on such organisms (Hance *et al.*, 2007; van Baaren *et al.*, in press). Data on the direct impact of heat stress at sub-lethal temperatures on the parasitoids' fitness are relevant in the face of global climatic warming.

Exposure to a sub-lethal temperature results in the death of weaker individuals. Insects that survive heat shock may pay the cost in their life history traits. Indeed, heat resistance may induce a number of physiological changes whose cost can be expressed by a reduction in reproductive output, a decrease or even delay in growth if immature stages are exposed, and/or changes in mating behaviour and in lifespan (Krebs & Loeschcke, 1994; Patton & Krebs, 2001; Rohmer *et al.*, 2004; Jørgensen *et al.*, 2006; Sisodia & Singh, 2006). However, the cost of resisting heat stress is not always a decline in life history traits. Indeed, previous studies have shown that stresses can produce a hormetic effect that generally increases longevity and can occur in males (Sørensen *et al.*, 2007) as well as in females (Lithgow *et al.*, 1995; Khazaeli *et al.*, 1997; Hercus *et al.*, 2003; Gomez *et al.*, 2009) and sometimes in both sexes (Scannapieco *et al.*, 2007). However, the hormesis of longevity may increase the cost of fitness by lowering fecundity due to trade-offs (Maynard Smith, 1958; Hercus *et al.*, 2003). Males and females can differ in their heat resistance, and the largest sex is generally the most resistant because a larger size resists dehydration better (Hadley, 1994).

Sometimes, heat shock in adults affects their progeny (parental effect), reducing the rate of egg hatching (Silbermann & Tatar, 2000) or inducing changes in morphology (Andersen *et al.*, 2005). However, other studies did not record any effect on progeny after exposing their parents to high temperatures (Hercus *et al.*, 2003; Huang *et al.*, 2007).

Here, we tested the impact of heat shock on the life history traits of adults of the aphid parasitoid *Aphidius avenae* Haliday (Hymenoptera: Aphididae) and on the later repercussions for their progeny. We simulated an unpredictable heat shock to which individuals were never before exposed. As this type of heat stress can provoke the death of half of the population, we hypothesized that it would affect the life history traits of the survivors. We measured the impact on mating ability, fecundity, adult lifespan and viability, developmental rates and the sex ratio (SR) of the progeny. Because *A. avenae* females are larger than males (Le Lann, unpublished data), we expected that females would be more resistant to heat stress than males. We also expected greater longevity (hormesis) and reduced fecundity because of an existing trade-off between these two traits in parasitoids (Ellers *et al.*, 2000). Finally, we hypothesized that

the progeny of heat-shocked individuals would have lower viability or would be affected in the time needed to develop due to a negative parental effect.

Material and methods

Insects

Aphidius avenae is an endoparasitoid of the grain aphid *Sitobion avenae* F. (Hemiptera: Aphididae). *Sitobion avenae* originated from a single parthenogenetic female (SA1 clone, INRA-zoology collection) collected in 1990 from a cereal crop near Rennes (Brittany, France). *Aphidius avenae* originated from *S. avenae* mummies collected from the same site in June 2006. Aphids and parasitoids were kept in Plexiglas boxes (50 × 50 × 50 cm) in climate rooms at 20 ± 1°C, 60 ± 10% RH and a 16L:8D photoperiod. Aphids were reared on winter wheat *Triticum aestivum*, cultivar 'Boston', provided by the Saaton Union Research Society (France). Each week, wheat plants infested by aphids were regularly introduced into the culture of parasitoids and honey was provided *ad libitum*.

To obtain standardized parasitoids for experiments, aphid mummies were collected from the culture and placed individually in gelatine capsules until the emergence of adult parasitoids. After emergence, the parasitoids were enclosed individually in micro-cages (L = 100 mm, Ø = 15 mm, with gauze at one end), containing moistened cotton and droplets of honey and were maintained in the climatic conditions mentioned above.

Heat exposure

To test the resistance of parasitoids to heat stress, we adapted the glass column designed by Powell & Bale (2006) (fig. 1). The parasitoids were introduced into the inner chamber using an aspirator. The air temperature of the inner chamber was controlled using an ethylene glycol stream heated in a thermostated bath (Haake K F3, Karlsruhe, Germany). The air temperature of the inner chamber was monitored using a thermal probe linked to a thermometer (sensitivity: 0.1°C) (Tempscan, Comark, Beaverton, Oregon, USA). Preliminary experiments showed that the temperature was equal throughout the column. We also recorded the relative humidity in the tube, which was constant at 50 ± 10%.

Twenty-seven male and 34 female *A. avenae*, each 24-h old, were tested for their resistance to heat exposure. All of the males (and in a second run, all of the females) were placed into the glass column at room temperature (≈20°C). The inside temperature was gradually increased from 25°C to 36°C at 1°C per minute, and then held constant at 36°C. The parasitoids are frequently exposed to this temperature during the summer near Rennes (source: Météo France). We terminated the heat exposure after 47 min (males) or 60 min (females) when 50 ± 10% of the parasitoids were considered to be dead because they showed no leg or antennae movement. This state corresponds to the heat stupor point, which is very close to lethal temperature in many insects (Vannier, 1994). The individuals were removed from the tube and observed every 30 min during 6 h to estimate the time of recuperation, if any. During this time, water and honey were placed near the survivors. Dead individuals were discarded, and the survivors were placed individually into micro-cages with water and honey. It took several hours

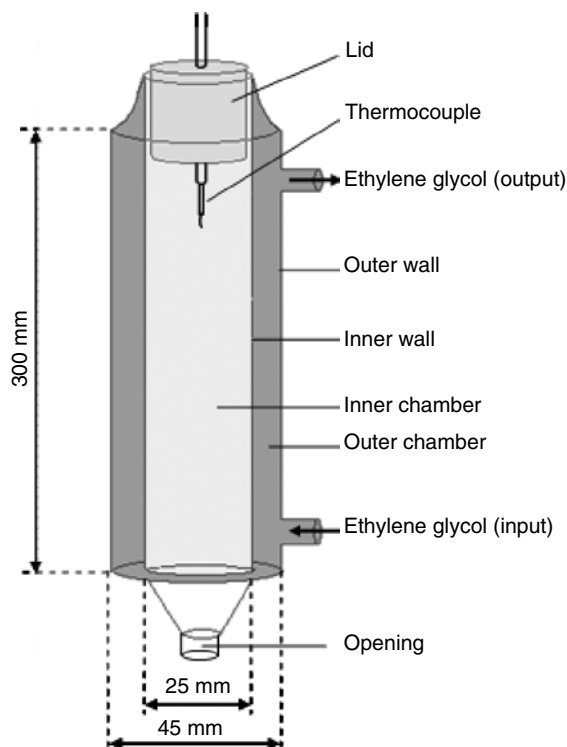


Fig. 1. Glass column used to expose the parasitoids to heat shock.

for the heat-shocked survivors to recover, during which time they remained motionless. They started to walk 4–6 h after exposure but were still unable to fly. After 24 h, the survivors completely recovered.

Mating

Twenty-four hours after heat exposure, the survivors (12 males and 12 females) were placed individually into glass tubes (L = 6.5 cm, \varnothing = 1 cm) with an untreated, 48-h-old individual (the same age as the survivors) of the opposite sex. When no mating occurred within 10 min, the untreated individual was removed and a new, untreated one was placed in the tube with the survivor. Untreated pairs were used as controls in the same conditions, i.e. 12 untreated females were placed into tubes with untreated males, and the males were replaced if no mating occurred. The same procedure was used for 12 untreated males that were placed into tubes with untreated females. A maximum of three partners was offered before we declared the tested individual unable to mate. Mating behaviour was video-recorded with a camera (Panasonic WV-PS03/C, Osaka, Japan) mounted on a binocular microscope (Olympus SZX-ILLD200, Center Valley, Pennsylvania, USA). The time spent in the tube before mating and the duration of the copulation were recorded (in seconds).

Oviposition and parasitism rate

After mating, each female (treated and control) was isolated individually for 24 h in a micro-cage containing one

wheat plantlet infested with 24 aphids. After 24 h, we placed the females individually into micro-cages with water and honey *ad libitum*. The parasitized wheat aphids were maintained in the climatic conditions described earlier.

Three to four days after the experiment, 12 aphids were chosen randomly and dissected in 70% ethanol under a binocular microscope and the parasitoid larvae counted to check for parasitism.

Length of development, viability and the sex ratio of the progeny and adult longevity

The 12 remaining aphids not selected to measure oviposition and parasitism rates were kept on wheat plantlets in a climate room until the mummification of the parasitized aphids. Each mummy was then isolated in a gelatine capsule until the parasitoid emerged. The numbers of mummies, their viability, the total time needed for the parasitoids to develop (from oviposition to the emergence of the adults) and the sex ratio (SR) of the progeny were registered.

The mortality rate of the adults used in the mating experiments (untreated and survivors) was checked twice each day to measure their lifespan.

Statistical analysis

All statistical analyses (Wilcoxon test (*W*) and *Chi*-square (χ^2)) were conducted with R version 2.4.0 (R Development Core Team, 2006).

Results

Mating and parasitism rate

Twenty-four hours after exposure, only 58.33% of the surviving females ($\chi^2 = 17.43$, $df = 1$, $n = 12$, $P < 0.001$; control 91.66%) and 50% of the surviving males ($\chi^2 = 4$, $df = 1$, $n = 12$, $P < 0.05$; control 75%) were able to mate successfully with an untreated individual. No significant differences were found in the time spent in the tube before mating (untreated pairs: 193 ± 41 ; untreated females with surviving males: 172 ± 64 , $W = 30.5$, ns; untreated males with surviving females: 139 ± 34 , $W = 32$, ns; mean \pm SE), nor in the duration of copulation between treatments (untreated pairs: 59 ± 3 ; untreated females with surviving males: 64 ± 4 , $W = 40$, ns; untreated males with surviving females: 60 ± 4 ; $W = 46$, ns; mean \pm SE). In pairs unable to mate, some males did not show any courtship behaviour (i.e. flapping their wings).

Surviving females that mated with untreated males parasitized fewer hosts and laid fewer eggs than untreated females that mated with untreated males, while untreated females that mated with male survivors did not show significant differences with untreated pairs (fig. 2a, b).

No significant differences were found in the mean number of mummies produced per female between the different treatments (untreated pairs: 6.7 ± 0.9 , $n = 76$; untreated females mated with surviving males: 5.2 ± 1 , $n = 31$, $W = 39$, ns; surviving females mated with untreated males: 6.4 ± 0.9 , $n = 64$, $W = 53$, ns; mean \pm SE).

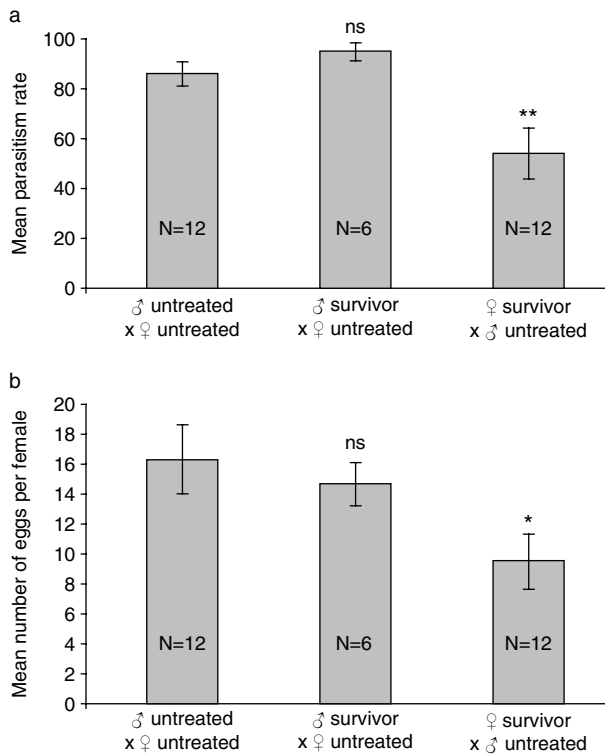


Fig. 2. (a) Mean parasitism rate per mated female (\pm SE); (b) Mean number of eggs laid per female (\pm SE) in the different types of mating situations set up between untreated individuals and survivors. The number of mated females (N) tested per treatment is indicated inside of the bars, ♂: males, ♀: females. Significance of the Wilcoxon test: * $P < 0.05$; ** $P < 0.01$; ns, not significant.

Viability, developmental rate and sex ratio of the progeny

The viability of the offspring of untreated pairs was significantly higher than for the offspring of female survivors (95.8% vs. 84%, respectively; $\chi^2 = 6.63$; $P = 0.01$) but not from the offspring of male survivors (95.8% vs. 89%, respectively; $\chi^2 = 2.31$; ns).

The untreated males emerged before the untreated females (protandry). In contrast, the female offspring of the surviving males emerged before the offspring of either sex from the untreated pairs, while the developmental rate for the other offspring from the surviving individuals did not statistically differ from their respective controls. The numbers of offspring obtained from male or female survivors did not significantly differ from the numbers of offspring obtained from the control pairs (fig. 3).

No differences were found in the offspring's sex ratio (untreated pairs: SR=0.37, $n=71$; offspring of male survivors: SR=0.27, $n=28$, $\chi^2 = 0.41$, ns; offspring of female survivors: SR=0.47, $n=25$, $\chi^2 = 0.34$, ns).

Lifespan

Untreated males and females had the same lifespan (22.6 ± 1.6 days ($n=12$) and 22.6 ± 1.7 days ($n=24$), respectively), but the longevity of the survivors was sex dependent.

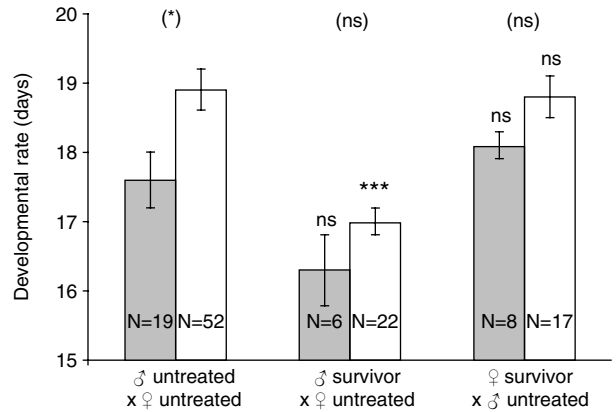


Fig. 3. Mean developmental rate (\pm SE) for the male (filled bars) and female (open bars) progeny of the parasitoids according to the different types of mating situations set up between untreated and surviving parents. The number of individuals (N) tested per treatment is indicated inside of the bars, ♂: males, ♀: females. The significance of the Wilcoxon test: *** $P < 0.001$; * $P < 0.05$; ns, not significant. Significant differences in development time between males and females are indicated in brackets.

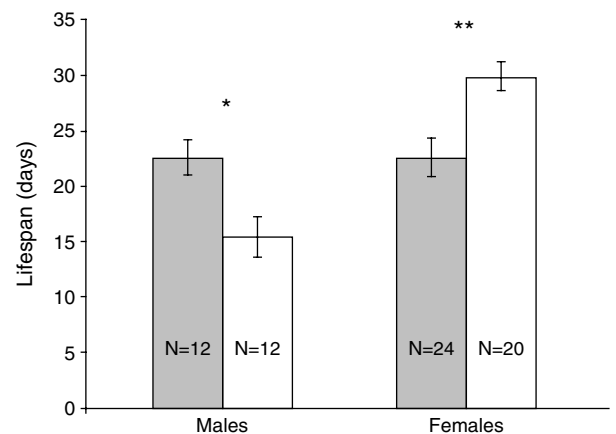


Fig. 4. Mean lifespan (\pm SE), of untreated (filled bars) and treated (open bars) male and female parasitoids. The number of individuals (N) tested per treatment is indicated inside of the bars. Significance of the Wilcoxon test: ** $P < 0.01$; * $P < 0.05$.

Male survivors lived approximately seven days less than untreated males (15.4 ± 1.8 days ($n=12$); $W = 116$, $P < 0.05$) and female survivors lived approximately seven days longer than untreated females (29.9 ± 1.3 days ($n=20$); $W = 358$, $P < 0.01$) (fig. 4).

Discussion

Our results show that after a sub-lethal heat shock that leads to about a 50% mortality rate, the fitness of the survivors is strongly affected. Moreover, the two sexes respond differently and their progeny is also affected. This response to stress by both adults and their progeny may have ecological consequences.

Impact of heat exposure on the fitness of survivors

Our results also show that the mating capacities of both sexes were affected after heat shock. Males and females can, thus, both be the cause of their mates' failures. Normally, virgin females produce pheromones that stimulate both upwind flight and elicit close-range courtship behaviour by males (McClure *et al.*, 2007). In our observations, we noted that some males did not show any courtship behaviour (i.e. flapping their wings). This might be due to anatomical injuries produced by the sub-lethal thermal stress that could affect muscular contractions, flight ability and fertility (Rohmer *et al.*, 2004; Krebs & Thompson, 2005). Patton & Krebs (2001) have also shown that after heat stress in male *Drosophila* sp., the heat shock proteins (HSP) in the thoracic muscles were lower than those in the head or in the abdomen, and that this is correlated with flight and courtship disruption. McNeil & Brodeur (1995) have shown that the courtship behaviour in males of the aphid parasitoid *Aphidius nigripes* is elicited by a short-range female pheromone consisting of cuticular lipids. In insects, these cuticular compounds are known to be altered by changes in temperature (Gibbs *et al.*, 1998). It has been shown, for example, that heat shock can alter the mating behaviour in *Drosophila* sp. (Markow & Toolson, 1990). So, the lack of courtship behaviour might be explained by changes in the production and/or dispersion of female pheromones, by the misdetection of pheromones by males or by the absence of or inefficient courtship by males. As both pheromones and courtship are essential to conspecific identification, and because mating with heterospecific individuals is costly, slight variations in these signals could easily result in a failure to mate and are counter-selected (Dobzhansky & Gould, 1982).

Surviving females laid fewer eggs, but survived seven days longer than the control females. The reduction in fecundity may be due to the direct cost of heat stress resistance as reported for *D. melanogaster* and for the parasitoid *T. carverae* (Krebs & Loeschcke, 1994; Scott *et al.*, 1997). However, as *A. avenae* tends to be synovigenic (i.e. can still mature eggs after their emergence) (Jervis *et al.*, 2001), the maturation of the eggs may have been interrupted by the heat stress induced at 24-h old, and the energetic resources that were not invested in egg production may then have been allocated to the lifespan of the female survivors. The possible hormetic effect observed in females (i.e. greater longevity after heat stress) is not uncommon (Lithgow *et al.*, 1995; Khazaeli *et al.*, 1997; Hercus *et al.*, 2003; Gomez *et al.*, 2009). Stronger females (that live longer) may also have been selected after heat shock.

Different impact on males and females

Our results show that females are more resistant to damage than males. Females have to be exposed to heat stress for a longer time to obtain a 50% mortality rate, and the lifespan of female survivors is longer than for male survivors. The better resistance to high temperatures in females is not unusual. This specificity may be due to a high concentration of HSPs in the ovaries and embryonic tissues and may explain why they suffer less damage than males (Palter *et al.*, 1986; Folk *et al.*, 2006; Krebs & Thompson, 2006). Moreover, a larger size is better for resisting dehydration (Hadley, 1994). Parasitoid females are larger than males,

which may explain their greater resistance to high temperatures. Also, the haplo-diploidy in parasitoids might be an important factor in differences in resistance between males and females. Indeed, females, with their double set of chromosomes, could be less sensitive to stress-induced damage to DNA; and diploid cells can repair damage through recombination. A combination of the three factors could be involved in this sex-dependant response.

Effect on the progeny of survivors

Heat shock in females seems to produce a significant decrease in the viability of mummies but not in the offspring of male survivors, which points to a possible maternal effect. Magiafoglou & Hoffmann (2003) studied the parental effect of cold shock in *Drosophila serrata*, for which also only the progeny of stressed females showed a lower rate of viability. Further studies are necessary to understand this difference in viability between the progeny of stressed males and females.

Our results show that the male progeny of the survivors develop more quickly than the male progeny of the control individuals, with, as a consequence, less protandry in stressed individuals. According to Quicke (1997), protandry is an adaptive trait in parasitoids because late-emerging males are likely to encounter only females that have already mated, and most female parasitoids mate only once. In *Aphidius ervi*, females are able to mate immediately after emergence, while males need several hours to become sexually mature. In such conditions, early-emerging males have a better chance of encountering virgin females and of increasing their probability of mating (He *et al.*, 2004). However, when adults are heat-exposed, the time between the emergence of both male and female progeny is significantly shorter than for the progeny of untreated adults. This may imply that males are not yet sexually mature when females emerge and could result in a male-biased sex ratio in the next generation due to the production of males by unmated females.

Ecological consequences of an unpredictable heat shock

Our results show three major consequences of heat stress lasting about one hour at 36°C. First, there was a high rate of mortality (around 50%). Second, there was a decrease in the fitness of the survivors. Finally, there was a lower level of protandry in the progeny, probably leading to a more male-biased sex ratio in the next generation. The rate of immediate mortality could also be increased by the behaviour of survivors in the hours following the stress. Indeed, after heat shock, *A. avenae* survivors were unable to move for several hours, something that makes them more vulnerable to predation.

The temperatures used in our experiments can be easily reached during heat waves (source: Météo France) in natural ecosystems, and the experimental length of exposure is relatively short in comparison to the duration of the maximum temperature during a day. Such a drastic reduction in reproductive output and the high rate of mortality observed could result in the severe crash of parasitoid populations. Even if parasitoids are able to survive because they certainly find shelter in cooler microhabitats during the hotter hours of the day, such results are good estimators of the impact of a higher incidence of heat

waves. Although it is certain that climate change will bring about changes in host-parasitoid systems, the precise outcomes are difficult to predict. Most models of host-parasitoid interactions predict an increase in pest outbreaks with climate change (Bezemer *et al.*, 1998; Cannon, 1998), which indicates that parasitoids may be less resistant than their hosts.

Further investigations are needed to elucidate the physiological mechanisms that underlie the changes produced by such heat stress on the fitness and life history traits of *A. avenae*.

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