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Stage- and sex-specific heat tolerance in the yellow dung fly *Scathophaga stercoraria*

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Abstract

Thermal tolerance varies at all hierarchical levels of biological organization: among species, populations, individuals, and even within individuals. Age- or developmental stage- and sex-specific thermal effects have received relatively little attention in the literature, despite being crucial for understanding thermal adaptation in nature and responses to global warming. We document stage- and sex- specific heat tolerance in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae), a species common throughout the northern hemisphere that generally favours cool climates. Exposure of eggs to temperatures up to 32°C did not affect larval hatching rate, but subsequent egg-to-adult survival at a benign temperature was reduced. Permanent transfer from benign (18°C) to hot temperatures (up to 31°C) at different larval and pupal stages strongly decreased egg-to-adult survival, though survival continuously improved the later the transfer occurred. Temporary transfer for only two days increased mortality more weakly, survival being lowest when temperature stress was imposed early during the larval or pupal stages. Adult flies provided with sugar and water tolerated 31°C longer than previously thought (5 days in males to 9 days in females). Eggs were thus less susceptible to thermal stress than larvae, pupae or adults, in agreement with the hypothesis that more mobile stages require less physiological protection against heat because they can behaviourally thermoregulate. The probability of mating, of laying a clutch, and hatching success were generally independently reduced by exposure of females or males to warm temperatures (24°C) during the juvenile and/or adult stages, with some interactions evident. High temperature stress thus affects survival differentially depending on when it occurs during the juvenile or the pre-reproductive adult life stage, and affects reproductive success via the mating behaviour of both sexes, female physiology in terms of oviposition, and fertility via sperm and/or egg quality. Our results illustrate that temperature stress, even when moderate and temporary, during early development can have profound lethal and non-lethal fitness-consequences later in life.

Keywords: Bogert effect; development; mating behaviour; reproduction; survival; temperature; thermal tolerance.

1. Introduction

Because of the general temperature dependence of all catalytic processes, temperature strongly affects the behaviour, physiology and life history of all organisms (Hochachka and Somero, 2002), and therefore ultimately the geographic distribution and abundance of species (Spicer and Gaston, 1999; Chown and Nicolson, 2004). Thermal tolerance varies at all hierarchical levels of biological organization: among species, populations and individuals, and even within individuals (Bowler and Terblanche, 2008). In particular, ontogenetic, age- or stage-, and also sex-specific variation within individuals have received relatively little attention in the literature, despite being crucial for understanding thermal adaptation of any particular organism in its natural context (Spicer and Gaston, 1999; Chown and Nicolson, 2004; Bowler and Terblanche, 2008).

Regardless of how it is estimated (see Angilletta, 2009), thermal tolerance is not expected to be constant over an individual's lifetime. This is particularly true for organisms with complex life cycles, such as insects, that are characterized by distinct ontogenetic stages encompassing eggs, larvae, pupae and adults (imago), which typically occur in different microhabitats that can vary substantially in temperature means and extremes (e.g., Krebs and Loeschcke, 1995; Vorhees and Bradley, 2012; Dierks et al., 2012). Such organisms may consequently be limited in achieving different stage-specific temperature optima within each of these environments, as tolerance at various developmental stages of an insect is likely linked, and thus potentially constrained, by common physiology and/or trade-offs (Huey and Kingsolver, 1993; Feder and Hoffmann, 1999; Angilletta, 2009). The extent to which species are able to cope with temperature extremes in different ontogenetic stages, and how exposure to such extremes during early ontogeny affects survival and reproduction also later in life, is of general interest for understanding the evolutionary potential and putative constraints of thermal adaptation (Bowler and Terblanche, 2008). For instance, studies of *Drosophila* not only revealed that short-term exposure to temperature during larval and pupal development

influences fly development and hatchability, but further that the resistance to temperature extremes can be somewhat uncoupled across developmental stages (Coyne et al., 1983; Krebs and Loeschcke, 1995; Crill et al., 1996), implying that different physiological pathways are recruited during ontogeny. These responses may or not be mediated by differential expression of heat shock proteins (hsp), patterns of which can be complex (Feder and Hoffmann, 1999; Hoffmann et al., 2003). Bowler and Terblanche (2008, p. 344) note a paucity of studies of age- and stage-dependent resistance especially to high temperatures in insects other than *Drosophila*. Examples include Davison's (1969) classic work on the blowfly *Calliphora*, Klok and Chown (2001) for kelp flies, Nyamukondiwa and Terblanche (2009) for fruit flies, or Belem Arias et al. (2011) for the flour beetle *Tenebrio*. Such studies indicate a trend towards a decline in thermotolerance with stage or age, possibly related to increasing mobility permitting behavioural compensation (cf. the Bogert effect discussed below), though so far the evidence is limited and inconclusive (Bowler and Terblanche, 2008).

A number of studies indicate that the sexes also differ in various ways in their vulnerability to temperature stress, with females being less sensitive than males at least in several cases. Sperm and egg production are affected by temperature (Blanckenhorn and Henseler, 2005), and studies on *Drosophila* and other species have reported differential effects of heat on spermatogenesis vs. oogenesis (David et al., 2005; Nguyen et al., 2013), male fertility vs. female fecundity (Krebs and Loeschcke, 1994), or in heat shock protein expression of males and females (Sørensen et al., 2007; Karl et al., 2009). Such sex-specific differences in heat sensitivity are critical because mating and the consequent reproductive success are per definition an interaction; in the extreme, infertility of one mating partner is sufficient to result in zero fitness of the other, ultimately contributing to population viability. An important concept in this context linking stage- and sex-specific thermotolerance is (heat or cold) hardening (a short-term response) or acclimation (a longer-term response), whereby

an individual can become more resistant or tolerant to extreme temperatures later in life (e.g., during the adult stage) by being exposed to them earlier in life (e.g., during the juvenile, egg, larval or pupal stage). Such carry-over thermal effects ultimately can increase survival and/or reproductive performance (i.e., fitness) of adult males and females (discussed generally by
5 Spicer and Gaston, 1999; Hoffmann et al., 2003; Chown and Nicolson, 2004; Bowler and Terblanche, 2008; e.g. Bubliy et al., 2013).

Following calls for more such work (Bowler and Terblanche, 2008), the main objective of our study was to document developmental stage- and sex- specific heat tolerance in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae), a commonly used
10 model species for studies of sexual selection, reproductive biology, and life history evolution (summarized in Blanckenhorn, 2009; Blanckenhorn et al., 2010). This widespread species is a frequent visitor of cow pastures that generally favours cooler climates. In the northern hemisphere, the species' distribution reaches latitudes up to Iceland and Spitzbergen (Sigurjónsdóttir and Snorrason, 1995), while in southern parts of their range these flies
15 preferentially occur at higher altitudes (Blanckenhorn, 1997). At the warm end of the thermal spectrum yellow dung flies are rather heat sensitive, which is reflected by the observation that adult flies largely disappear from the pastures during the hot summer, apparently to hide in the cooler forest in some state of reproductive quiescence (Parker, 1970; Gibbons, 1987; Blanckenhorn et al., 2001). This interpretation is supported by experimental laboratory and
20 field studies estimating the fly's optimal developmental temperature to be ca. 23°C (Blanckenhorn, 1999) and repeatedly documenting that temperatures above 25°C kill larvae, pupae (Blanckenhorn, 1997, 1998) and adults (Ward and Simmons, 1990; Reim et al., 2006), while sub-lethally high developmental temperatures lead to the phenotypic expression of hidden genetic variation in female reproductive morphology in form of a fourth spermatheca
25 to store sperm that is rarely expressed in the field and most likely originates from

developmental instability in response to unnaturally high metabolic activity (Berger et al., 2011; Schäfer et al., 2013).

We report a total of four separate laboratory experiments (Fig. 1) assessing heat-mediated mortality of yellow dung fly eggs, larvae, pupae, and adults. While adult mortality in response to high temperatures has been investigated before in this species in different ways (Ward and Simmons, 1990; Reim et al. 2006), the role of temperature stress during earlier developmental stages has not yet been studied in detail even though this is crucial for understanding thermal adaptation in a broader life-history context. Based on the life history of the yellow dung fly (Blanckenhorn, 2009; Blanckenhorn et al., 2010) we can generate some predictions. Although females adjust oviposition behaviour according to temperature to some extent in the field (Ward et al., 2002; Blanckenhorn et al., 2012; Demont et al., 2012), eggs should be most heat tolerant, as they are laid into dark-coloured, heat-absorbing fresh cow dung on open pastures, thus exposing them to hot temperatures potentially exceeding 30°C for at least one day (until larval hatching). Hatched larvae forage in the fresh dung, periodically surfacing for air, and eventually pupate in the dung or in cooler microhabitats in the ground underneath the dung pat. Based on this background we thus can expect a decline (of whatever form) in the heat tolerance of yellow dung flies from the egg, via the larval and pupal to the adult stage, as the mobile adult and larval stages can behaviourally thermoregulate whereas the eggs have to be physiologically prepared to cope with hot temperatures (the so-called Bogert effect: Huey et al., 2003; Bowler and Terblanche, 2008; Mitchell et al., 2013).

With an additional, fifth experiment (Fig. 1) we investigate the sex-specific effect of hot (24°C) vs. optimal (18°C) temperature experienced by males and/or females during the juvenile or adult stage on their reproduction (copulation, oviposition, and fertility) in a fully factorial design. In this anautogenous (income-breeding) species, gonads are developed

during the juvenile (larval and especially pupal) stage, but the predatory adults need protein from prey to produce eggs and sperm during a pre-reproductive adult stage lasting about one week in males and two weeks in females (Blanckenhorn and Henseler, 2005). By way of pairings in all possible combinations, we aimed at attributing effects of heat on reproductive success to developmental (in the juvenile) vs. physiological/behavioural (in the adult) factors in either sex, thus investigating within-species acclimation effects.

2. Materials and methods

For the purposes here, we assessed the most drastic effects of constant high temperatures on mortality at overabundant larval food (i.e., dung, thus avoiding juvenile crowding), as well as effects on reproductive behaviour and physiology in terms of copulation, oviposition and fertility. While adult body size was measured in some (but not all) of our experiments, temperature effects on morphology following the temperature-size rule (smaller when warmer) are well known in this species and have been treated elsewhere (Blanckenhorn, 1997; Hosken et al., 2000; Blanckenhorn and Henseler, 2005; Reim et al., 2006; Kjaersgaard et al., 2013). Adult flies were originally collected on a farm in Fehraltorf, near Zürich, Switzerland (N47°23', E8°44'), and held for 2 to 4 generations in the laboratory using previously described methods (Blanckenhorn et al., 2010) before being used in our experiments.

Temperature effects on egg mortality (Experiment 1)

To assess the effect of hot temperatures on survival of the egg and the larva developing therein (Fig. 1), clutches of females ($N = 30$) mated with one male that were raised and held at 20°C, a benign and optimal temperature environment, were split among five temperatures (12°C, 17°C, 22°C, 27°C, 32°C) right after being laid at room temperature (ca. 10 eggs per

temperature). These temperatures are well within the range of temperatures measured in the field over three spring and autumn seasons, with temperatures in the shade ranging from 9°C in mid March 2003 to 24°C in September 2004, while temperatures under direct sunlight (not specifically measured) are considerably higher. The eggs were transferred onto a piece of wet
5 filter paper, which was then placed into a small plastic container with an abundant amount of ca. 20 g of fresh cow dung so that larval hatching could be scored. After larval hatching, the dung containers were transferred to constant 20°C until all adult offspring flies emerged. We used a logistic model with binomial errors to analyze survival of the individual eggs as a function of temperature treatment, additionally entering the mother's identity (i.e., family) as
10 a random effect.

Temperature effects on larval and pupal mortality (Experiments 2 & 3)

To assess the effect of high temperatures on survival during the larval and pupal stages, we exposed flies developing at 18°C to either 25°C, 28°C or 31°C, additionally including a
15 non-exposed 18°C control treatment (Fig. 1). In one experiment, this heat treatment lasted for 2 days only (whereafter the flies were transferred back to 18°C), and took place either during the early larval stage (day 2 after egg laying), the late larval stage (day 7), the early pupal stage (day 12), or the late pupal stage (day 17; cf. Blanckenhorn et al., 2010). In the second experiment, the flies were transferred permanently to one of the three hot temperatures at the
20 specified developmental stage.

In both these experiments, full clutches laid by females mated with only one male, both raised and held at 18°C, were split into four batches of ca. 15 eggs and randomly assigned to one of the four treatment times. There were replicate clutches ($N = 12$ to 15) of different mothers per temperature (i.e., a crossed factor), each split among the four treatment times
25 (i.e., a nested factor). We scored survival as the proportional number of emerged adults given

the number of eggs that entered each family container. We used separate logistic models for both experiments to analyze the number of emerged (i.e., survived) flies as a function of temperature treatment and time point as fixed factors, again entering the mother's identity (i.e., family) as a random effect.

5

Temperature effects on adult mortality (Experiment 4)

Mortality of yellow dung fly adults at high temperatures has been investigated previously in the laboratory at 27°C without food or water (Ward and Simmons, 1990) and at 25°C with water only ("starvation resistance": Reim et al., 2006). Here we estimated the ability of adults of both sexes to survive at 31°C (i.e., their lifespan) when held individually in 100 ml glass bottles and supplied with water and sugar (Fig. 1). We used the flies emerging from the 18°C control treatment of the juvenile experiment described above. We employed Cox regression to analyze these survival data, as we scored adult longevity after emergence.

15 *Sex-specific effects of juvenile or adult exposure to hot temperature on reproduction (Experiment 5)*

In order to assess the effect of hot temperature on adult reproduction, the full-sib offspring of a randomly formed pair were raised family-wise (for ca. 3 weeks) as juveniles in a dung container at either benign (18°C) or hot (24°C) temperatures in a large climate chamber (Fig. 1). Adult males and females emerging from any family container were 20 randomly split upon emergence into the same two temperature environments to obtain flies of both sexes that experienced one of the four possible juvenile/adult temperature combinations (18°C/18°C; 18°C/24°C; 24°C/18°C; 24°C/24°C). The adult temperature treatment lasted ca. 2 weeks, after which time males and females from the four treatments were randomly paired 25 in all possible combinations. We scored three reproductive traits: whether or not copulation

occurred (binary variable; for both sexes), whether females subsequently laid a clutch of eggs (binary variable; females only), and the fertility of this clutch (proportion of adult offspring emerged from the clutch; an interactive trait potentially affected by both the male and the female). Individual females were sequentially confronted with up to three males of the same temperature treatment combination within a few days to assure a sufficient sample, and only when a female did not copulate and/or lay after this time she was scored as non-reproducing. All males and females were mated only once during the experiment. We measured hind tibia length of all flies as an index of body size and took note of the adult age of all flies at the time of copulation, to control statistically for potential size and age effects within treatment combinations.

We analyzed these data as a four-way logistic general linear model with binomial errors, with the juvenile and adult temperature treatment of both males and females as fixed crossed factors, and hind tibia length and male and female adult age as covariates. We additionally entered (i.e., controlled for) family identity as a random effect in the model, although here we were not particularly interested in any possible genetic effects on reproduction. In the end we obtained $N = 253$ matings, sample sizes ranging from a minimum of $N = 44$ for males of the 24°C/24°C to $N = 79$ for females of the 18°C/18°C juvenile/adult temperature treatment combination, and only $N = 135$ (i.e., slightly more than half) of these females produced a clutch for which hatching success could be scored.

3. Results

Temperature effects on egg mortality (Experiment 1)

Larval hatching rate averaged between 85 – 90% and was not significantly affected by temperature (logistic model with binomial errors and family as a random effect: Wald $\chi^2 = 1.51$, $df = 4$, $P > 0.3$; Fig. 2, top). Individuals were treated with the experimental temperatures

only during the egg phase and later transferred to benign 20°C for the remainder of their development; subsequent larva-to-adult mortality varied with egg rearing temperature (Wald $\chi^2 = 13.29$, $df = 4$, $P = 0.01$) in a quadratic fashion (polynomial contrast: Wald $\chi^2 = 12.43$, $df = 1$, $P < 0.001$; Fig. 2, bottom). Particularly larvae from eggs exposed to 32°C showed reduced overall larva-to-adult survival.

Temperature effects on larval and pupal mortality (Experiments 2 & 3)

When exposing developing flies at four different life stages to hot temperature pulses for two days, overall survival decreased from ca. 72% to 59% as the temperature pulses increased from 25°C to 31°C (Wald $\chi^2 = 16.27$, $df = 3$, $P = 0.001$), while the developmental stage of transfer was marginally non-significant ($\chi^2 = 6.59$, $df = 3$, $P = 0.086$; temperature by stage interaction was non-significant ($\chi^2 = 13.61$, $df = 9$, $P = 0.137$) and hence removed from the model; Fig. 3, left). However, post-hoc comparison indicated that survival was lower when the hot temperature pulse was applied early vs. late during the larval or pupal stage ($\chi^2 = 8.80$, $P = 0.003$).

Analogous permanent transfer to the same hot temperatures at the different developmental stages (however without the 18°C control) resulted in a much stronger decline in survival from ca. 35% to 5% as temperature increased from 25°C to 31°C ($\chi^2 = 28.42$, $df = 2$, $P < 0.001$), and here also a strong effect of the stage of transfer, with survival continuously increasing from ca. 8% to 28% the later the transfer occurred ($\chi^2 = 17.73$, $df = 3$, $P < 0.001$; the temperature by stage interaction was again not significant ($\chi^2 = 5.60$, $df = 6$, $P = 0.469$) and hence removed from the model; Fig. 3, right).

Assuming equal sex ratio as the null expectation, there was no sex bias in juvenile mortality in either experiment (overall proportion of females emerged: 0.519 ± 0.031 (SE)).

Temperature effects on adult mortality (Experiment 4)

Cox regression showed that the larger males died significantly sooner than the females when held permanently at 31°C with water and sugar only (Wald $\chi^2 = 35.86$, $df = 4$, $P < 0.001$). Of all adult females (total $N = 169$) that had emerged from the 18°C control treatment of the above experiment, 50% survived 9.1 days (95% CI: 7.7, 12.7) of exposure to 31°C (LD50 estimated by probit analysis), while for males (total $N = 157$) this number was only 5.1 days (1.4, 6.5).

Sex-specific effects of juvenile or adult exposure to hot temperature on reproduction

(Experiment 5)

Copulation probability increased with female age, relating to gamete maturation (Wald $\chi^2 = 13.23$, $df = 1$, $P < 0.001$; total $N = 253$). More interestingly, copulation probability was lower when females were exposed to hot temperature (24°C) as juveniles ($\chi^2 = 7.03$, $df = 1$, $P = 0.008$) or as adults ($\chi^2 = 9.55$, $df = 1$, $P = 0.002$; main effects only, interaction not significant; Fig. 4a). The pattern looked similar albeit weaker for males, as the reduction of copulation probability was only significant when male adults ($\chi^2 = 5.41$, $df = 1$, $P = 0.02$) but not juveniles ($\chi^2 = 1.16$, $df = 1$, $P = 0.284$) were exposed to hot temperature (Fig. 4b). All interactions, male or female body size, and male age were not significant and dropped from the final model.

The probability of producing a clutch was reduced strongly when females were exposed to hot temperature (24°C) as adults ($\chi^2 = 10.92$, $df = 1$, $P = 0.001$; total $N = 253$), and more weakly when they were exposed to hot temperature as juveniles ($\chi^2 = 3.88$, $df = 1$, $P = 0.05$; Fig. 5a). Fewer clutches were also produced in response to male temperature treatment, interestingly only when males were exposed to hot temperature as juveniles **and** raised as adults at the benign temperature (18°C) (male juvenile by male adult treatment interaction:

$\text{Chi}^2 = 8.87$, $\text{df} = 1$, $P = 0.003$; Fig. 5b). Lastly, there was also a female adult temperature by male juvenile temperature interaction, as fewer clutches were laid only when male juveniles and female adults were exposed to hot temperature ($\text{Chi}^2 = 8.82$, $\text{df} = 1$, $P = 0.003$; Fig. 5c). All other interactions, male or female body size or age were not significant and dropped from the final model.

Fertility (i.e., hatching success) was reduced when females were exposed to hot temperature as juveniles ($\text{Chi}^2 = 4.71$, $\text{df} = 1$, $P = 0.03$) but not as adults ($\text{Chi}^2 = 0.92$, $\text{df} = 1$, $P = 0.338$; total $N = 135$; Fig. 6a). The same holds true for males (juvenile temperature effect: $\text{Chi}^2 = 8.27$, $\text{df} = 1$, $P = 0.004$; adult temperature effect: $\text{Chi}^2 = 0.12$, $\text{df} = 1$, $P = 0.739$), although there additionally was a marginally non-significant male juvenile by male adult temperature treatment interaction ($\text{Chi}^2 = 3.04$, $\text{df} = 1$, $P = 0.081$), as male adult exposure to 24°C reduced hatching success when the male experienced benign temperatures of 18°C as a juvenile, but adult exposure to hot temperatures did not decrease male fertility further when the male had already experienced 24°C during the juvenile phase (Fig. 6b). All other interactions, male or female body size or age were not significant and dropped from the final model.

4. Discussion

Our study documents that the effects of high temperatures on mortality and reproductive behaviour in the yellow dung fly *Scathophaga stercoraria* are developmental stage- and sex-specific in a manner that can be reasonably reconciled with this insect's life history in the wild. The data further illustrate that high developmental temperatures, even when occurring over relatively short periods of time, can have profound fitness consequences later in life. Below we discuss these aspects in more detail.

Stage-specific effects of high temperatures on mortality

Adult and juvenile yellow dung flies were previously known to be heat sensitive, as throughout the northern hemisphere this species favours cooler climates at high altitudes and latitudes, and because flies disappear from the pastures in summer due to mortality or migration to cooler habitats (summarized in the Introduction and Blanckenhorn et al., 2010).

Adults were previously shown to frequently die at temperatures above 25°C (Ward and Simmons, 1990), and starvation resistance with water only increased with body size (Reim et al., 2006). Nevertheless, yellow dung fly eggs should be able to endure temperatures exceeding 30°C when laid into fresh cow dung pats on open pastures exposed to sunshine, as argued in the Introduction. We therefore expected a decline in the heat tolerance from the egg, via the larval and pupal to the adult stage, based on the simple intuitive argument that behavioural thermoregulatory plasticity should allow more mobile (stages of) ectotherms to avoid extreme temperatures, thus making physiological thermoregulatory plasticity less necessary (the Bogert effect: Huey et al., 2003; Mitchell et al., 2013). The story turned out not so simple.

First, contrary to previous assessments without nutrients (Reim et al., 2006) or without even water (Ward and Simmons, 1990), we found here that pre-reproductive adult flies provided with sugar (their primary energy source) and water, but without prey (their source of protein for reproduction), can tolerate very hot temperatures of 31°C for about 5 (males) to 9 (females) days on average, longer than previously thought. Second, although eggs could indeed tolerate 32°C well with larvae still hatching properly, this treatment did increase subsequent juvenile mortality by 10-15% relative to exposure to temperatures $\leq 27^\circ\text{C}$, even after larvae were moved to the benign temperature of 20°C (Fig. 2). Such carry-over effects to later life stages of detrimental egg temperatures have also been reported in other species (reviewed by Bowler and Terblanche, 2008; e.g. Zhang et al., 2014). Third, permanent

transfer to temperatures $\geq 25^{\circ}\text{C}$ strongly reduced survival of juveniles to much less than 40% of the 18°C control level unless the treatment commenced late during the pupal phase (Fig. 3); permanent transfer to 31°C during the larval and early pupal stages in fact resulted in nearly 100% mortality. In contrast, a two-day heat pulse of 25°C or 28°C lowered ($< 5\%$) survival only slightly, while a 31°C pulse reduced survival by 30-40% when it occurred during the early larval or early pupal stage, but only by ca. 15% when it occurred during the late larval stage, and essentially no extra mortality was induced when the transfer occurred during the late pupal stage (Fig. 3). As has become increasingly appreciated, substantial and long-lasting fitness decrements can result from relatively short exposure to heat extremes (Krebs and Loeschcke, 1994, 1995; Hoffmann et al., 2003; Bubliy et al., 2013; Zhang et al., 2013; Zhang et al., 2014). In yellow dung flies, the effect of heat stress during the juvenile phase is thus complex and definitely not linear, being stronger early during both the larval and pupal stages but weaker during the late larval and pupal stages. While it can be easily understood why effects are only slight very late during the pupal phase, at which time metamorphosis should be essentially completed, the non-linear effect earlier during juvenile development is more difficult to interpret. We suspect that the early larval stages, when most of the growth occurs (Teuschl et al., 2007), as well as the early pupal stage, when metamorphosis begins, are the most demanding and developmentally sensitive phases in terms of energy and oxygen (Pörtner, 2001; Klok et al., 2004) requirements or the potential of cell damage (e.g., Wang et al., 2007), at least relative to the later stages of larval and pupal development. Complex life stage-dependent hsp expression is another possibility mediating these differences (Feder and Hoffmann, 1999; Hoffmann et al., 2003; e.g. Belen Arias et al. 2011). Unfortunately we cannot address these various plausible underlying mechanisms further, as we did not assess any physiological parameters here.

Overall therefore, our experiments with yellow dung flies did not support a simple

relationship of heat tolerance with the mobility of the life stage, as conjectured by Bowler and Terblanche (2008) in accordance with the Bogert effect (which was also not found by e.g. Davison, 1969, or Krebs and Loeschcke, 1995). We concede that it is difficult to directly compare the various mortality effects quantitatively because the developmental stages, and also our treatments, had very different durations, which moreover depended on temperature. What remains is that the egg is less sensitive than the following life stages, as originally predicted, probably indeed because this stage cannot behaviourally thermoregulate and therefore needs to be physiologically protected against insolation in some way. We thus conclude that yellow dung flies probably have not physiologically evolved stage-specific thermal tolerance in an adaptive manner in response to shifting costs and benefits. Rather, it seems more parsimonious that mortality at a given stressfully high temperature is a direct proximate response to increased demands during these particular phases of development.

Sex-specific effects of hot temperatures on reproduction

Yellow dung flies are nutritionally anautogenous income breeders (Foster, 1967). This means that gonads are developed during the juvenile larval and especially pupal stages, while the gametes are first produced during a pre-reproductive adult stage lasting about one week in males and two weeks in females, and continuously thereafter (Blanckenhorn and Henseler, 2005). Temperature stress during the juvenile and adult phases therefore can independently affect reproduction in this species, which is what we found. Females exposed to (relatively hot) 24°C as juveniles or adults were less likely to copulate at a given age. This was qualitatively similar albeit weaker for males, with adult heat exposure showing stronger effects than juvenile exposure. As females and males are not receptive or able to copulate initially during early adulthood due to lack of gametes (Blanckenhorn and Henseler, 2005;

Blanckenhorn et al., 2007), we here again also confirmed that females were more likely to copulate as they aged.

The pattern for female oviposition was more complex. Females were less likely to produce a clutch of eggs (at a given age) when exposed to 24°C as adults, and more weakly so when they were exposed to 24°C as juveniles. Fewer clutches were also produced when males were raised at 24°C as juveniles *and* subsequently held at benign 18°C as adults, a counterintuitive interactive effect that we have difficulties explaining (Fig. 5b). Particularly few clutches were produced when females were exposed to high 24°C as adults and their male partners as juveniles (Fig. 5c). Egg maturation of adult females is delayed if not halted at hot summer temperatures, and male gonad (during the juvenile stage) and subsequent gamete development (during the pre-reproductive adult stage) is also compromised by high temperatures (Blanckenhorn and Henseler, 2005), in the extreme leading to male infertility. The latter may result in abnormal male mating behaviour, which females may be able to use as a cue for not laying eggs. For instance, Janowitz and Fischer (2011) reported a comparable response but opposite in direction in *Bicyclus* butterflies: females laid more eggs when copulating with a heat-stressed male, perhaps a sign of terminal investment. However, similar patterns are also expected if males adjust their mating propensity depending on female reproductive status. For example, context dependent male-mating has been implicated in a recent study of alternative mating tactics in the yellow dung fly, where small-sized males in an orchard away from the dung pat showed higher mating propensities and were more discriminatory in response to female egg development status, in contrast to the standard situation at the dung pat where intense sexual competition generally favours large body size in males (Gress et al., 2014).

Fertility (i.e., hatching success) of laid eggs was reduced when females were exposed to high temperature as juveniles but not as adults. The same holds true for males, supporting our

above interpretation that hot temperatures during the juvenile stage carry over and compromise male sperm quality in the adult stage (see also Nguyen et al., 2013). Male adult exposure to 24°C also reduced hatching success, but only when the male had experienced benign temperatures during the juvenile phase and not when he was exposed to hot temperatures previously. This suggests some heat hardening or acclimation effect in response to high juvenile temperatures, perhaps mediated by hsp (Hoffmann et al., 2003; Bublly et al., 2013).

In summary, hot temperatures have differential lethal effects at different life stages in yellow dung flies, eggs being less susceptible than larvae, pupae and adults. Hot temperatures also have multi-faceted and sometimes long-lasting non-lethal effects on dung fly reproduction, depending on whether they occur during the juvenile or the pre-reproductive adult life stage. Temperature stress affects the mating behaviour of both sexes, female physiology in terms of oviposition, and probably also male physiology in terms of sperm production, as well as fertility via sperm and/or egg quality, although we did not directly measure any physiological traits in this study. We also note that we here only tested constant temperatures, even though fluctuating temperatures are more natural and certainly can produce differing results (Hoffmann et al., 2003; see e.g. Kjaersgaard et al., 2013). We conclude that behavioural thermoregulation of all mobile stages contributes substantially to temperature tolerance of yellow dung flies in accordance with their life history, ultimately allowing these flies to persist under stressfully hot conditions in the field (cf. Mitchell et al., 2013). As the consequences of temperature variation depend on when and by how much temperature varies, determining and predicting the ensuing population dynamics will therefore be complex and challenging.

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Fig. 1. Schematic summary of the various experiments performed to assess stage- (1 – 4) and sex-specific (5) heat tolerance in the yellow dung fly *Scathophaga stercoraria*.

Fig. 1

Fig. 2. Mean $\pm$ SE yellow dung fly egg survival (i.e. larval hatching success) at various temperatures (top), and subsequent egg-to-adult survival at benign 20°C (bottom).

Fig. 2

Fig. 3. Mean $\pm$ SE egg-to-adult survival (i.e., adult emergence success) of yellow dung flies when transferred at various juvenile life stages either for 2 days only (left) or permanently (right) from benign 18°C (control) to increasingly stressful hot temperatures (25°C, 28°C, or 31°C).

5

Fig. 3

Fig. 4. Mean $\pm$ SE copulation probability after (a) female and (b) male exposure to benign (18°C) and hot (24°C) temperatures during the juvenile (x-axis) or adult life stage (18°C: circles and hatched lines; 24°C: squares and solid lines).

Fig. 4

Fig. 5. Mean $\pm$ SE probability of laying a clutch after (a) female or (b) male exposure to benign (18°C) and hot (24°C) temperatures during the juvenile (x-axis) or adult life stage (18°C: circles and hatched lines; 24°C: squares and solid lines). (5c) depicts the interaction between male exposure as juveniles and female exposure as adults.

5

Fig. 5

Fig. 6. Mean $\pm$ SE proportion of offspring emerged from clutches laid (i.e., hatching success or fertility) after (a) female or (b) male exposure to benign (18°C) and hot (24°C) temperatures during the juvenile (x-axis) or adult life stage (18°C: circles and hatched lines; 24°C: squares and solid lines).

Fig. 6