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# Life stages of an aphid living under similar thermal conditions differ in thermal performance

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ABSTRACT

Heat responses can vary ontogenetically in many insects with complex life cycles, reflecting differences in thermal environments they experience. Such variation has rarely been considered in insects that develop incrementally and experience common microclimates across stages. To test if there is a low level of ontogenetic variation for heat responses in one such species, the English grain aphid *Sitobion avenae*, basal tolerance [upper lethal temperature ($ULT_{50}$) and maximum critical temperature ($CT_{max}$)], hardening capacity ($CT_{max}$) and hardening costs (adult longevity and fecundity) were measured across five stages (1st, 2nd, 3rd and 4th-instar nymphs and newly moulted adults). We found large tolerance differences among stages of this global pest species, and a tendency for the stage with lower heat tolerance to show a stronger hardening response. There were also substantial reproductive costs of hardening responses, with the level of stress experienced, and not the proximity of the exposed stage to the reproductive adult stage, influencing the magnitude of this cost. Hence hardening in this aphid may counter inherently low tolerance levels of some life stages but at a cost to adult longevity and fecundity. Our findings highlight the significance of ontogenetic variation in predicting responses of a species to climate change, even in species without a complex life cycle.

## 1. Introduction

Climate change is expected to alter the distribution and phenology of ectotherms including herbivorous insects in the coming decades (Bale et al., 2002; Hoffmann et al., 2013; Kingsolver et al., 2013). An increasing frequency and intensity of extreme temperature events (IPCC, 2013) may be particularly challenging for many ectotherms (Kingsolver et al., 2013; Ma et al., 2015; Overgaard et al., 2014; Vasseur et al., 2014). Small insect herbivores often have short life cycles (Danks, 2006) leading to overlapping generations (Gullan and Cranston, 2005), so any life stage may experience heat stress. As a consequence, the response of these insects to heat stress will likely depend on the thermal sensitivity of all stages (Gilchrist et al., 1997), and population-level effects may vary depending on the distribution of life stages in a population (Zeigler, 2013). This makes it important to understand stage-specific thermal responses (Bowler and Terblanche, 2008; Kingsolver et al., 2011).

Basal tolerance and plastic responses both contribute to the ability of ectotherms to counter heat stress (Fischer and Karl, 2010; Hoffmann et al., 2013). The former represents an inherent and evolved level of tolerance independent of environmental conditions. Plastic responses provide organisms with the capacity to cope with environmental variation within or across generations and involve physiological changes which enhance survival under temperature extremes following exposure to less extreme conditions; it may involve hardening (following exposures of a few hours) or acclimation (longer exposures of days or even weeks) but no genetic changes (Hoffmann et al., 2003). For many species with complex life stages, stage-specific environmental pressures may lead to variation in tolerance responses across life stages; stages which experience chronic stress might be expected to show higher basal tolerance (Sørensen et al., 2001) and life stages exposed to fluctuating conditions might be expected to show increased plastic responses (Rinehart et al., 2006). Overall, less mobile life stages (e.g. egg or pupa) are expected to be more tolerant according to the Bogert effect (Huey et al., 2003; Marais and Chown, 2008), reflecting differences in the microclimate experienced (Kingsolver et al., 2011; Krebs and Loeschcke, 1995) which may change selection pressures for heat tolerance and plasticity (Mitchell et al., 2011).

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In contrast to the species with stage-specific features, aphids and many other small hemimetabolous insects do not have an immobile pupal stage, and nymphs resemble adults for which similar thermal conditions might usually be shared (Gullan and Cranston, 2005). In addition, there is a similar heating rate across ontogeny given their small body size (Angilletta, 2009; Huey and Bennett, 1990). Therefore, a similar pattern of selection for heat resistance across stages is expected. Despite some previous studies on aphids focusing on life stage variation in heat tolerance (Ma et al., 2004a; Piyaphongkul et al., 2012) and plastic responses (Harrison and Barlow, 1973; Ju et al., 2011), it is unclear whether these small insects developing incrementally between the juvenile and adult stage show reduced variation for stress tolerance and plastic capacity when compared to hemimetabolous insects.

In addition to affecting fitness directly, heat stress may produce costs associated with plastic thermal responses which have been explored in many species and especially in insects with a complex life history (Krebs and Loeschcke, 1994; Scott et al., 1997; Silbermann and Tatar, 2000). For most studies focusing on the adult stage, reproductive costs after hardening have been confirmed (Krebs and Loeschcke, 1994; Roux et al., 2010; Scott et al., 1997; Zhang et al., 2013). Low costs of hardening may occur across multiple stages given the modularity of metamorphosis insulating later stages from the disturbance in early development (Potter et al., 2011) or compensatory growth counteracting the negative effects of poor early environment (Dmitriew and Rowe, 2007; Orizaola et al., 2010). Consistent with these expectations, there was little impact on fecundity when stress was imposed on the egg stage (Potter et al., 2011; Xing et al., 2014, 2015), or various life stages (Knapp and Nedvěd, 2013; Zani et al., 2005). In the case of *P. xylostella*, costs increased with stress dosage and proximity of exposure to the adult stage (Zhang et al., 2015) which suggests that repair during metamorphosis and/or compensation may be important in this species. For hemimetabolous insects lacking metamorphosis, the gradual development between nymphs and adult increases the likelihood of impacts of stress imposed on various life stages on adult performance. This raises the question of whether these two types of insects show different cost patterns across stages.

Here we test the hypothesis that life stages of a small hemimetabolous insect experiencing a similar microclimate have similar levels of tolerance to heat stress, by considering basal tolerance and hardening responses in the English grain aphid, *Sitobion avenae* (Fabricius). During growth period, this species has an anholocyclic life cycle (Fig. S1) with a short life cycle (reach maturity within 1 week) and high reproduction (50–60 offspring per adult) (Asin and Pons, 2001). A mother always deposits nymphs in a 'group' which can persist about a week, and even more mobile older and apterous individuals tend to move only a few meters (Dean, 1973) where the microclimate is likely to be similar. The aphids instead spend time and resources processing food to ensure high rates of growth (Dixon, 1998). Therefore, life stages of *S. avenae* usually experience similar thermal microhabitat during a growing season (Fig. S2), and daily maximum temperatures they experience can be quite high and reach 35 °C (e.g. Fig. S3) which is regarded as stressful for this species (Kieckhefer et al., 1989).

We address the following questions. (1) Do basal thermal tolerances and hardening capacities vary between stages? (2) Is there a relationship between basal thermal tolerance and hardening capacity across stages? (3) Do large reproductive costs of hardening responses occur at the adult stage, and do these depend on stage that has been stressed? We consider two measures of basal tolerance [upper lethal temperature (ULT) and maximum critical temperature ($CT_{max}$)] in five stages (1st, 2nd, 3rd and 4th-instar nymphs and newly moulted adult). Furthermore, we investigate hardening effects on $CT_{max}$ and consider longevity and fecundity to assess costs. The results indicate that basal tolerance, hardening response and costs associated with a brief heat exposure vary with ontogenetic stage. Lower heat resistance and stronger hardening responses were associated, while costs expressed in terms of longevity and fecundity were determined by the period of the hardening rather than the stage exposed.

## 2. Materials and methods

### 2.1. Stocks and rearing

Stocks and rearing conditions followed Zhao et al. (2014). English grain aphids were collected from a winter wheat field near Beijing (39°48 N, 116°28 E), and were then reared on 10–20 cm tall winter wheat seedlings in screened cages (60 × 60 × 60 cm) at 22 ± 0.5 °C, 50–60% relative humidity, and a photoperiod of 16 L : 8 D. Seedlings were replaced every week. Experiments were undertaken after this stock had been reared under these conditions for 2 years.

### 2.2. Tested insects

Preliminary experiments were performed to determine the developmental time of the 1st to 4th-instar nymphs of *S. avenae* at a 22 °C rearing temperature, so that cultures could be set up to produce a range of developmental stages for testing. Developmental time was about two days for the 1st or 4th-instar nymphs, whereas the development of the 2nd and 3rd -instar nymphs each required about 1.5 days. Therefore, all stages were obtained by allowing newly emerged nymphs (< 6 h old) to develop different times of 0, 2, 3.5, 5 and 7 days to obtain newly born nymphs (< 6 h old, 1st-instars), 2nd-instars, 3rd-instars, 4th-instars and newly moulted adults respectively.

At the beginning of the rearing period, insects were placed individually in a rearing tube consisting of a 5 ml plastic tube (diameter 15 mm, length 55 mm) covered with nylon gauze (200 mesh size), with 0.6% agar solution in one-third of the tube holding a newly-excised wheat leaf. Aphids were transferred using a fine camel-hair brush and rearing tubes were replaced on the third day (in the cases of 4th-instar nymphs and adults).

### 2.3. Experimental design

#### 2.3.1. Experiment 1: upper lethal temperatures of stages

$ULT_{50}$ values were determined using a method modified from Hazell et al. (2010). Preliminary experiments were performed to identify a suitable range of test temperatures for each stage. Mortality typically increases from 10% to 100 % with 2 °C changes in *S. avenae*. Consequently, temperatures were assessed at 0.2 °C increments within the range 38.4–41.4 °C depending on the stage tested.

Instar stages were tested separately at various time points but originated from the same generation. For each state, at a particular temperature, 8–12 developmentally-synchronized aphids were placed into a 5 ml plastic tube plugged by a cotton-wool ball (6 tubes per temperature). These six tubes were held in place by a multiporous lucite plate (100 × 60 mm) and placed into a programmed glycol bath (Ministat 230-cc-NR; Huber Ltd., Germany, accuracy ± 0.01 °C), keeping the cotton-wool balls in tubes lower than the external liquid. Bath temperature was held at 22 °C for 10 min and then heated at 0.5 °C $min^{-1}$. Once the test temperature was reached, it was held for 10 s to reduce the potential effect of a time lag (Walsberg and Wolf, 1996), and then reduced at 0.5 °C $min^{-1}$. Once the temperature had returned to 22 °C, all aphids were transferred from the glycol bath to recovery arenas (90 mm transparent plastic dish, covered by nylon mesh) at 22 °C and fed with fresh wheat leaves. Survival was assessed 24 h after heat treatment. The process was repeated to produce a survival curve for each stage (n = 698, 670, 659, 658 and 411 individuals for the 1st, 2nd, 3rd, 4th-instar nymphs and adult, respectively). Sixty individuals untreated controls per stage group were also set up (these were not exposed to the ramping treatment).