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Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress?

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ABSTRACT

Thermal stress at one life stage can affect fitness at a later stage in ectotherms with complex life cycles. Most relevant studies have focused on extreme stress levels, but here we also show substantial fitness effects in a moth when pupae are exposed to a relatively mild and sublethal heat stress. We consider the impact of a 35 °C heat stress of 2 h in three geographically separate populations of the oriental fruit moth (OFM, *Grapholita molesta*) from northern, middle and southern China. Heat stress negatively affected fecundity but increased adult heat resistance and adult longevity. Fitness effects were mostly consistent across populations but there were also some population differences. In the Shenyang population from northern China, there was a hormetic effect of heat on female longevity not evident in the other populations. Adults from all populations had higher LT_{50}s due to heat stress after pupal exposure to the sublethal stress. These results highlight that the pupal stage is a particularly sensitive window for development and they have implications for seasonal adaptation in uncertain environments as well as changes in pest dynamics under climate warming.

1. Introduction

One of the most notable effects of global climate warming is an increase in the incidence of hot events (IPCC, 2013). Although a single hot event may not increase daily temperatures enough to impact the generation time of ectotherms (Cannon, 1998; Harrington et al., 2001), high temperatures for a few hours can nevertheless have negative effects on adult performance and offspring survival (Liang et al., 2014; Zhang et al., 2013). Extreme high temperatures are therefore likely to influence demographic rates (Ma et al., 2015), particularly in ectotherms with complex life cycles where stages may have inherently different sensitivities to temperature (Kingsolver et al., 2011; Zhao et al., 2017). Although extreme thermal conditions near lethality (e.g. close to 40 °C for many Lepidoptera) can influence fitness across life stages (Marchioro and Foerster, 2011; Tofangsazi et al., 2012; Zhang et al., 2015b), less extreme increases in temperature may also have effects, and such temperature increases are becoming more common. For instance, in northern and southern China, the frequency of daily maximum ambient temperatures exceeding 35 °C has increased in the June to August period over the last ten years (see Liang et al., 2014; Zhang et al., 2015b, Zhang et al., 2013). These periods of sublethal heat stress may have cross-stage effects (Zhang et al., 2015b), particularly if they coincide with a sensitive life stage.

Life stages of insects with complex lifecycles often inhabit different microhabitats, which in turn can lead to variation in thermal sensitivities and other traits that are important for responses to climate (Kingsolver et al., 2011; Pincebourde and Casas, 2015; Potter et al., 2011; Woods, 2010). For example, holometabolous insects might be buffered against temperature fluctuations at the pupal stage if they pupate in the soil, whereas larvae on plants and dispersing adults are likely to be exposed to much more variable conditions, resulting in different thermal sensitivities (Chown and Nicolson, 2004; Kingsolver et al., 2011; Zhang et al., 2016a). Different physiological and behavioral mechanisms may be involved when life stages adapt to such variable climatic conditions (Kingsolver et al., 2011).

When a particular life stage is exposed to stressful thermal conditions, stress effects may have impacts across life stages, such that exposure at one stage results in (usually) detrimental effects on fitness at a later stage (Bader and Williams, 2012; Kingsolver and Huey, 2008). For instance in moths, chronic heat stress of 30 °C during some life stages alters adult demographic rates and reproductive patterns (Zhang et al., 2015b). A heat stress of 40 °C experienced at a developmental stage

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close to the adult stage can have particularly strong negative effects on fitness in moths (Zhang et al., 2015a). However, it has been reported that heat stress early in life can cause an increase in lifespan, due to hormesis occurring when a mild stress is encountered at an early critical stage (Olsen et al., 2006; Le Bourg et al., 2001). Hormesis is described as a variety of stimulatory responses to low doses of stress which improve longevity and other fitness performance measures (Neafsey, 1990; Rattan, 2008). The timing and level of stress are critical for triggering hormesis effects, which may involve a range of physiological and molecular mechanisms (Costantini et al., 2010). Understanding the nature of these effects is important when predicting the negative effects of warm periods.

In this study, we characterized the consequences of a mild heat stress during the pupal stage of oriental fruit moth (OFM, *Grapholita molesta*) on the subsequent fitness of adults and their offspring. OFM is one of the world's most invasive pest insects of stone and pome fruits, causing severe economic loss in the fruit industry globally (Kirk et al., 2013; Zhang et al., 2016b). OFM larvae stay in fruit until they mature at the 5th instar larval stage when they pupate at the bottom of fruit and are therefore exposed to air temperatures. Because these temperatures will vary depending on geographic location, we consider responses in multiple populations of OFM. In general, responses of insects to thermal stress can vary across geographic populations, since the optimal temperature for development, thermal breadth and thermal limits of populations often strongly affected by local ambient environmental temperatures (Griffiths et al., 2005; Sunday et al., 2011). For instance, in *Drosophila melanogaster*, clines in heat and cold resistance match predictions based on average temperature variation from coastal eastern Australia (Hoffmann et al., 2002; Sgrò et al., 2010). Such patterns can both reflect the results of natural selection and plastic changes induced by the environment, which are recognized as important for maximizing fitness in an organism that inhabits variable environments (Hoffmann et al., 2013; Kingsolver et al., 2016; Overgaard et al., 2011; Terblanche et al., 2006).

To capture geographic variation in China, we consider OFM from three locations (Table 1) which experience gradual temperature increases from May to September, but within a distinct temperature zone.

To test cross-stage effects, we exposed pupae to 35 °C and considered differences in adult longevity, fertility and heat resistance. We use these data to examine: (1) whether this sublethal heat stress on pupae affects adult longevity, reproduction or heat resistance; (2) whether there were also effects of the pupal stress on offspring hatch rates; and (3) whether these fitness effects differed across populations reflecting genetic differentiation in OFM.

2. Materials and methods

2.1. Stocks

Three populations of OFM used in this study were sampled from Shenyang (North, N41.64°, E123.52°), Baoding (middle, N38.98°, E115.07°) and Guangzhou (South, N24.49°, E114.56°) in China in 2014 (Table 1). Each population was established from > 100 adults emerging from > 300 field larvae and maintained subsequently with several hundred individuals. They were reared on fresh Fuji apples held in transparent containers in the laboratory at 24 °C and 60% RH under a photoperiod of 15:9 (L:D). Once the fully developed larvae emerged from apple, they were collected and checked daily until they built cocoons within the containers. To establish patterns of differentiation between the populations, developmental time from egg to pupation and pupal weight were measured in the absence of heat stress before the experiments began. To reduce the potential for laboratory adaptation confounding results, all experiments were completed within three generations of laboratory rearing of OFM.

2.2. Temperature stress and physiological responses

Stress. To examine adult performance after sublethal heat stress during the pupal stage, OFM pupae from the three populations were collected on the third day of pupation in glass vials and exposed to 35 °C in a water bath for 2 h. Normally, OFM take five days after pupal formation to develop to the adult stage under the laboratory conditions used here, thus the third day is the mid-pupal stage. The treatment of 35 °C for 2 h was chosen for two reasons: 1) it represents a day temperature and duration time frequently encountered during summer in northern and southern orchards in recent years; and 2) this temperature treatment did not influence emergence rate, emergence time or result in morphologically abnormal adults in pilot experiments. In contrast, higher temperatures in pilot experiments (e.g. 40 °C) resulted in negative effects on pupal survival and development. Over 600 pupae per population were exposed to the pupal heat stress, while controls were reared at the same time without heat stress.

Longevity and fecundity. After adults emerged, the longevity of both unmated female and male adults held in glass vials individually was recorded. Moths were provided with 5% honey solution on a cotton ball on top of a bottle, and the cotton ball was changed daily. Fifty individuals of each sex were used from each population per treatment.

Lifetime fecundity was scored on groups of moths containing equal numbers of males and females. Five pairs of females and males were held together in bottles and at least six biological repeats per population were set up for each of the heat and control treatments. All the adults received 5% honey solution on a cotton ball at the top of a bottle for nutrition. The diameter of > 80 eggs randomly selected per treatment was measured by using a micro-objects measurement system (Oriental Farmer's Biotechnology Co., Ltd in Beijing) which functions like an ocular micrometer. The numbers of hatched eggs on the wall of bottles were counted daily until all adults in a bottle had died.

Heat tolerance. To determine adult heat resistance after pupal exposure to heat stress, we measured the survival rate of newly emerged adults (equal numbers of female and male) after they were exposed to 42 °C for 3, 4, 5, 6 and 7 h. Exposure to 42 °C for 1 or 2 h did not result in any mortality in pilot experiments. Between 30 and 48 individuals

Table 1
Temperatures and precipitation during May to September in three geographical populations. Mean maximum is daily mean maximum, and mean minimum is daily mean minimum for each month downloaded from the China Meteorology Data Sharing Service System from 1996 to 2015.

Population(latitude N)	Variable	May	June	July	August	September
Shenyang	Mean maximum (°C)	23	27	29	28	24
(N41.64°)	Mean minimum (°C)	11	17	21	19	12
	Precipitation (mm)	54	92	187	166	75
Baoding	Mean maximum (°C)	27	32	32	30	27
(N38.98°)	Mean minimum (°C)	15	20	23	21	16
	Precipitation (mm)	32	67	161	150	48
Guangzhou	Mean maximum (°C)	30	31	33	33	32
(N24.49°)	Mean minimum (°C)	23	25	25	25	24
	Precipitation (mm)	289	300	227	233	188