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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Stage-specific heat effects: timing and duration of heat waves alter demographic rates of a global insect pest

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Abstract The frequency and duration of periods with high temperatures are expected to increase under global warming. Thus, even short-lived organisms are increasingly likely to experience periods of hot temperatures at some point of their life-cycle. Despite recent progress, it remains unclear how various temperature experiences during the life-cycle of organisms affect demographic traits. We simulated hot days (daily mean temperature of 30 °C) increasingly experienced under field conditions and investigated how the timing and duration of such hot days during the life cycle of *Plutella xylostella* affects adult traits. We show that hot days experienced during some life stages (but not all) altered adult lifespan, fecundity, and oviposition patterns. Importantly, the effects of hot days were contingent on which stage was affected, and these stage-specific effects were not always additive. Thus, adults that experience different temporal patterns of hot periods (i.e., changes in timing and duration) during their life-cycle often had different demographic rates and reproductive patterns. These results indicate that we cannot predict the effects of current and future climate on natural populations by simply focusing on changes in the mean temperature. Instead, we need to incorporate the temporal patterns of heat events relative to the life-cycle of organisms to describe population dynamics and how they will respond to future climate change.

Keywords Hot event · Temperature · *Plutella xylostella* · Carry-over effect · Adult lifespan · Reproduction

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Introduction

Temperature is one of the most important climatic factors influencing the survival (Hoffmann et al. 2003, 2012), development and growth rates (Kingsolver and Huey 2008; Forster and Hirst 2012) and reproduction (Geister and Fischer 2007; Berger et al. 2008) of ectotherms. As such, temperature is increasingly recognized as a key factor driving the dynamics and distributions of natural populations (Savage et al. 2004; Paaijmans et al. 2013; Ma et al. 2015b). However, temperature is rarely constant; instead, it fluctuates, and organisms can experience short or prolonged periods of hot (or cold) days. Importantly, the frequency and duration of periods with hot days has increased around the globe in recent decades [e.g. Europe (Beniston 2004), Australia (Pezza et al. 2012), Russia (Trenberth and Fasullo 2012), China (Huang et al. 2010)], and this trend is expected to continue under global warming (IPCC 2013). As an example, the frequency of hot days with a daily mean temperature of ≥30 °C in summer has increased during the last decade near Wuhan in China (Fig. 1a). As a consequence, even short-lived organisms are increasingly likely to experience periods with hot temperatures at some point of their life-cycle. Furthermore, due to inter-annual variation in the timing and duration of hot days, heat events may affect a single life-stage or multiple life-stages, with

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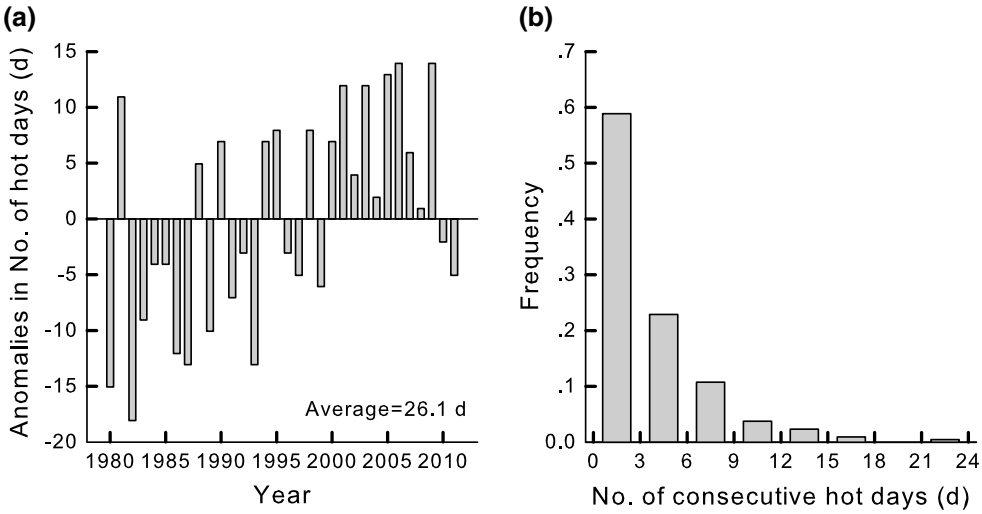

Fig. 1 Number of hot days (daily mean temperature ≥30 °C) in June–August at Wuhan (30.62N, 114.13E) from 1980 to 2011. **a** Anomalies in the number of hot days (relative to the average temperature for 1980–2011), **b** frequency of consecutive days of a hot event. Daily mean temperature records were downloaded in 2013 from the China Meteorological Data Sharing Service System

the stages affected varying among years. Despite recent progress in this field (Bowler and Terblanche 2008; Kingsolver et al. 2011; Bozinovic and Portner 2015), it remains unclear how this variation in the stage-specific timing of hot events affects key demographic rates of organisms.

Demographic effects of hot periods are likely to be stage specific due to thermal tolerance (Bowler and Terblanche 2008; Zhang et al. 2015), and the physiological response to temperature (Folguera et al. 2010; Forster and Hirst 2012) typically change during the ontogeny of organisms. For example, adult amphibians and reptiles are able to tolerate higher temperatures for a longer time than those at earlier life-stages (e.g. eggs or larvae) (Sherman 1980; Xu and Ji 2006; Telemeco 2014), while in invertebrates, juvenile stages often have a higher tolerance (Krebs and Loeschcke 1995; Klok and Chown 2001; Ma et al. 2004a; Lyons et al. 2012; Zhang et al. 2015). It is also possible that the effects of temperatures experienced during early life-stages could also be carried over to alter adult traits. Temperature experiences during larval stages could impact adult size (Kingsolver and Huey 2008) and reproduction (Hodin and Riddiford 2000; Bader and Williams 2012), while those during metamorphosis could impact the formation of internal and external reproductive structures (French et al. 1998; Stevens 2004), which might lead to substantial damage to adult reproduction. However, persistent carry-over effects of exposure to hot temperature at early life-stages do not always occur (Huey et al. 1995; Potter et al. 2011; Xing et al. 2014). Exposure to high temperatures during the adult stage may also limit mating performances (Katsuki and Miyatake 2009; Mironidis and Savopoulou-Soultani 2010), egg maturation (Berger et al. 2008) and oviposition pattern (Berger et al. 2008; Zhang et al. 2013), which in turn could alter the temperature effects during early life-stages.

To predict how variation in the timing and duration of hot days during the life-cycle of an organism influences demographic rates we need to determine whether stage-specific temperature effects are independent of each other (i.e. additive). Do negative effects of high temperatures during the larval, pupal and adult stage sum up to determine the effects on adult reproduction, or are the effects more or less than expected from the individual life-stage effects? The modular life-cycle of insects allows repair and even restructuring (in case of holometabolous species) of morphology, hormone regulation systems and physiological metabolism (Consoulas 2000; Consoulas et al. 2002; Seifert et al. 2012). Such changes could amplify or reduce the effect of hot temperatures on subsequent life-stages, as demonstrated by Huey et al. (1995) who exposed Drosophila larvae to increased temperatures and found no effect on egg production and fecundity patterns. Furthermore, if high temperatures only occur during part of the life-cycle, there would be sufficient time for the individuals to recover from potential heat damage during the other life-stages (Moran 1994; Potter et al. 2011; Stoks and Córdoba-Aguilar 2012). This suggests that not only the timing, but also the duration of high temperature events during the life-cycle of an individual could have important consequences for demographic rates and adult fecundity. Given that individuals can experience hot periods during one, multiple, or all life-stages, answering this question is essential to predict how organisms respond to changes in temperature regimes and how they will perform under future climate change scenarios.

In the study reported here we adopted an experimental approach to test whether and how the timing and duration of high temperature events during the life-cycle of an organism affects reproductive performance. Our questions were: (1) Could the impact of increased temperature in early life-stages carry over to alter adult fitness? (2) Are the effects stage specific? (3) Do these carry-over effects depend on temperature conditions in subsequent life-stages (i.e. independent or additive)?. Our results demonstrate that the effects of high

temperature on our test organism were typically stage specific and that temperature effects could carry over into the adult stage. In addition, heat events during multiple life-stages were not always additive. These results suggest that shifts in the timing and duration of hot periods during the life-cycle of organisms can alter the demographic rates of individuals and potentially alter population dynamics.

Materials and methods

Study organism

We use the diamondback moth *Plutella xylostella*, the most destructive pest of cruciferous crops around the world, as our model organism. Under the optimal temperature regime for growth and development (24–26 °C), the larval, pupal and adult stages last approximately 8.4–10, 3–4.9 and 12.2–14.2 days, respectively, and at constant high temperatures (either 29 °C or 30 °C), they last approximately 6–8.9, 2.6–4.5 and 9.0–10.5 days, respectively (Chung et al. 1989; Ma and Chen 1993; Dan et al. 1995; Liu et al. 2002). *P. xylostella* has a high thermal tolerance and can survive and develop at 30 °C (Ma and Chen 1993; Shirai 2000; Liu et al. 2002). However, such high temperatures (i.e. >28 °C) are usually associated with a reduction in reproduction (Ma and Chen 1993; Dan et al. 1995). In our study site (30.62N, 114.13E) at Wuhan, China, peak densities of *P. xylostella* usually occur from early May to early June and consist of multiple overlapping generations. During peak periods, all life-stages may be present in the field at the same time; thus, a hot event (>30 °C) during this period can concomitantly affect the larval, pupal and/or adults stages. In addition, because of the short life-cycle of this species and variations in the length of hot periods (Fig. 1b), hot periods can affect a single stage, multiple stages, or even the whole life-cycle of *P. xylostella*. Finally, ovariole development and egg maturation of *P. xylostella* may begin before adult eclosion (Castelo Branco and Gatehouse 1999; Justus and Mitchell 1999), suggesting that adverse conditions experienced prior to the adult stage could have negative effects on reproduction.

Stock rearing

Individuals used in the experiment originated from a field population collected during May in 2008. About 400 larvae and pupae (5–10 individuals per cabbage) were collected from a cabbage field (50 × 50 m) in the Experiment Station of Hubei Academy of Agricultural Sciences (30.62N, 114.13E) in Wuhan, China. The captive population was reared on an artificial diet at 25 ± 1 °C, 30–40 % relative humidity (RH) and a photoperiod of 15:9 h (light:dark), as described by Zhang et al. (2013).

Experimental design

We identified the independent and combined effects of increased temperature during three developmental stages on adult traits using a 2 × 2 × 2 full-factorial design with two levels (25 or 30 °C) each of larval temperature (LT), pupal temperature (PT) and adult temperature (AT) of *P. xylostella* (Fig. 2). First, we divided 1200 newly hatched

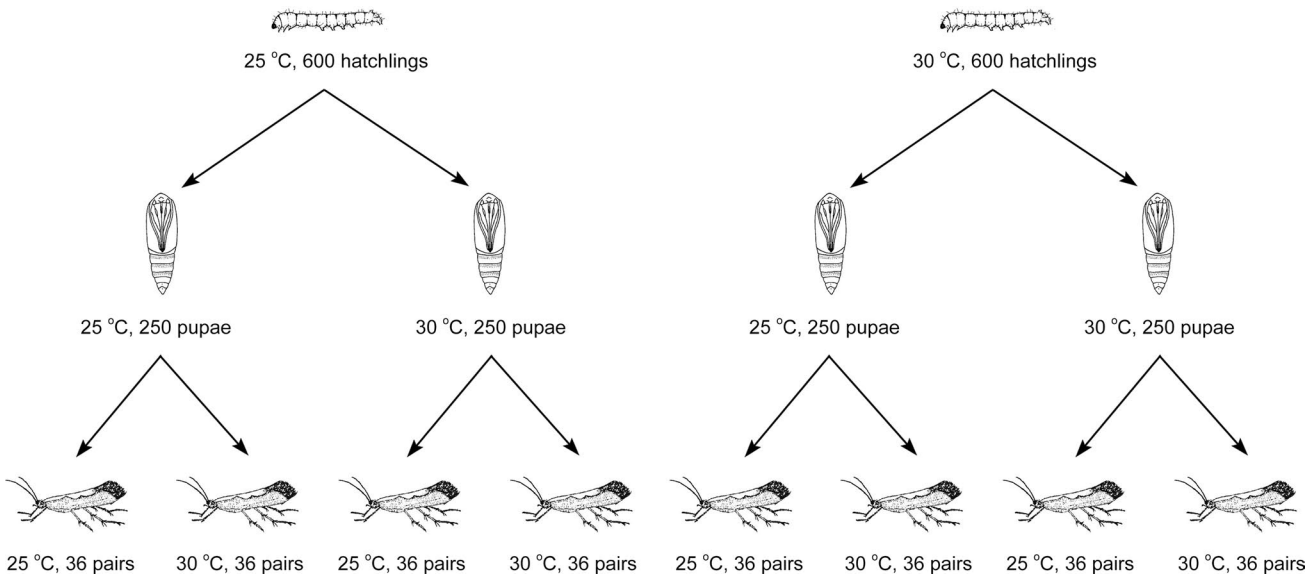

Fig. 2 Schematic flowsheet of experimental design showing the stage-specific temperature treatments. In the 2 × 2 × 2 factorial design, the temperature (25 vs. 30 °C) was independently manipulated during the larval, pupal, or adult stage within the life-cycle of *Plutella xylostella*

larvae (≤2 h old) evenly into two groups, with each group assigned to one of two temperature treatments (25 or 30 °C). Once larvae reached pupation (larval development: 9.5 days at 25 °C; 6.5 days at 30 °C), pupae were then randomly assigned to one of the two temperature treatments. After the pupal stage (4.5 days at 25 °C; 3.5 days at 30 °C), a total of 36 newly emerged (1-day-old) males and females from a given temperature treatment were paired, and each pair was again randomly assigned to one of the two temperature treatments (=8 treatment × 36 pairs = 288 pairs in total). Larvae and adults were reared in glass bottles (length 15 cm, diameter 3 cm), while pupae were reared individually in glass tubes (length 10 cm, diameter 1.5 cm). Larvae were reared in groups of 50 per bottle and received 4 g of artificial food (Southland Products Inc., Lake Village, AR). Food was renewed every 3 days to assure that development and growth were not food limited. Adults received a cotton ball soaked with a 5 % honey solution and a piece of "egg card" made of Parafilm (American Can Co., Greenwich, CT) for egg-laying. The cotton balls and egg cards were replaced daily. All treatments were kept at 30–40 % RH and a photoperiod of 15:9 h (light:dark). Temperature and humidity in the chamber were monitored with HOBO loggers (Pro V2 Temp/RH Data Logger U23-001; Onset Computer Corp. Bourne, MA), and temperature variation for all treatments was ±1 °C.

Response variables

After pairing, we checked adult survival and counted eggs (on the wall of the glass tube and egg card) daily until the adults died. To determine whether differences in pupal weight could explain observed differences in adult reproduction, we selected 90 new pupae randomly from each of two larval treatments and weighed them individually.

Statistical analyses

To test how variation in the timing and duration of hot temperatures influences adult survival, we used a Cox proportional hazard model to analyze how temperature treatments affected male or female longevity using the "coxph" function in the package "survival" in R (Therneau 2014). All two- and three-way interaction effects were not significant (and did not improve the model fit) and were thus removed from the final analyses. Subsequent temperature conditions were found to be able to influence the carry-over effect by prior thermal stressors. To examine how temperature during larval or/and pupal stage influenced the longevity of both male and female adult *P. xylostella* at different temperatures when adults, we used generalized linear models (GLMs) at different adult temperatures with four combinations of larval and pupal temperature as fixed (continuous) effects, followed by Duncan's post hoc multiple comparisons between treatments.

We then examined how treatments affected reproduction by testing whether and how treatments affected total fecundity (i.e. total numbers of eggs produced per female), pre-oviposition period, oviposition period and post-oviposition period. We defined pre-oviposition period as the number of days before females started ovipositing (after being paired with males), taken as an indication of delayed onset of reproduction. However, because there was virtually no delay in reproduction (only 7 of 281 females delayed oviposition by 1 day), there was insufficient variation to meaningfully analyze the data. Oviposition period was considered to be the number of days during which females laid eggs, indicating how long females were reproductively active. Post-oviposition period was taken as the number of days between the final oviposition event and the death of a female and was interpreted as "reproductive senescence", i.e. the period when females stopped reproduction before they die. During the experiment, three females did not lay any eggs (for larval/pupal/adult temperature treatments: 1 at 30/25/25 °C, 2 at 30/25/30 °C). Data from treatments in which no eggs were laid were removed from the fecundity and oviposition analyses; including these treatments did not alter the general results but did decrease the model fit. For all of the above analyses we used GLMs with larval, pupal, and adult temperature and all their interactions as fixed effects followed by Duncan's post hoc multiple comparisons. To determine whether differences in lifetime fecundity were simply driven by female longevity, we repeated the analysis but included longevity as a fixed, continuous variable in the GLM model. A quatratic term was required to account for the significant non-linear relationship between longevity and fecundity or oviposition period. As we found no significant interaction between longevity and other fixed factors, we dropped these interactions from the final analysis.

To gain a more detailed insight into the temporal patterns of egg production, we used a repeated measures generalized linear mixed model (GLMM) with eggs/day as the dependent variable, larval, pupal, and adult temperatures as fixed effects and individual identity as the random effect to account for the non-independence of observations over time. Comparisons of Akaike Information Criterion values indicated that an unstructured covariance matrix to account for repeated observations best fit the data. This analysis demonstrated that in treatment differences were largely driven by relative differences in early (first 2 days) versus late (>3 day) egg production.

To determine whether differences in adult traits were driven by differences in size, we used a *t* test to test for LT effects on pupal weight. All statistical tests were either performed using SAS (SAS Institute, Cary, NC) or R (R Development Core Team 2013).

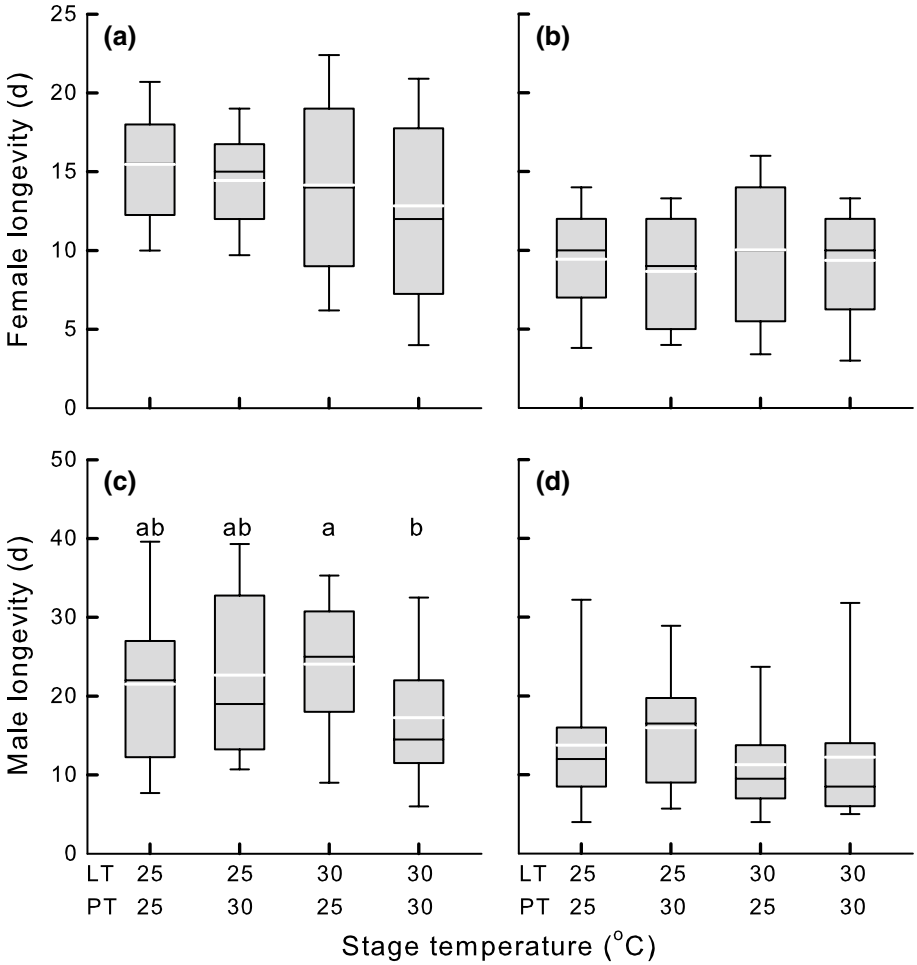

Fig. 3 Box plot of the longevity for both sexes of *P. xylostella* according to different larval and pupal temperature treatments. Longevity of females kept at 25 °C (**a**) or 30 °C (**b**) and males at 25 °C (**c**) or 30 °C (**d**) in the treatments involving the combination of larval temperature (*LT*) and pupal temperature (*PT*). The *upper* and *lower boundary* of the box indicates the 75th percentile and 25th percentile of the data set. The *black* and *white horizontal line* within the box represents the median and mean values. *Error bars* above and below the box indicate the minimum and maximum values. *Different lowercase letters* above each box indicate significant differences ($P < 0.05$) between the four treatments

Results

Survival and longevity

The probability of survival of females ($Z = 8.61$, $P < 0.0001$) and males ($Z = 6.32$, $P < 0.0001$) declined by almost twofold when adults experienced high temperatures. As a consequence, the mean longevity of females and males declined by 4.7 and 8.3 days, respectively, and the maximum lifespan decreased by 10 and 5 days, respectively (Fig. 3). Temperature experienced during the larval ($Z = -0.81$, $P = 0.416$) or pupal stage ($Z = 1.40$, $P = 0.163$) had no significant effect on female survival. Male survival was not affected by temperature during the pupal stage ($Z = -0.28$, $P = 0.779$), but it was reduced by 12 % at higher larval temperatures, although this was marginally non-significant ($Z = 1.90$, $P = 0.077$). Post hoc comparisons showed that exposing both the larval and pupal stages to 30 °C significantly shortened the mean longevity of males by >4.3 days at 25 °C (Fig. 3c; $P = 0.022$), but not the longevity of adult males when they were also exposed at 30 °C (Fig. 3d; $P = 0.137$). This result indicates that heat exposures during the larval and pupal stages can carry over to influence male survival, but only if adult males are not exposed to high temperatures as well.

Lifetime fecundity

Lifetime fecundity was significantly reduced by 13.9, 8.7 and 19.1 % at high larval, pupal and adult temperatures, respectively (Table 1; Fig. 4). We did not observe any significant interactions among temperature treatments (Table 1). However, exposing both larval and pupal stage to 30 °C significantly decreased the total fecundity by >14 % when the adult stage was exposed to 25 °C (Fig. 4a; $F_{3,\,135} = 4.99$, $P = 0.003$), but not when exposed to 30 °C (Fig. 4b; $F_{3,138} = 1.50$, $P = 0.219$). Fecundity also increased significantly (non-linearly) with adult lifespan. After accounting for differences in lifespan, adult temperature was no longer significant, but the significant effect of larval temperature remained [Electronic Supplementary Material (ESM) Table S1]. This result suggests that the negative effect of adult temperature on fecundity was

Table 1 Effects of stage-specific temperature treatments on adult reproductive traits of *Plutella xylostella* at the larval, pupal and adult life-stages and their interactions

Treatment	Lifetime fecundity (eggs/female)		Oviposition period (days)		Post oviposition period (days)	
	F value	*P*	*F* value	*P*	*F* value	*P*
LT	11.2	0.001*	7.75	0.005*	8.36	0.004*
PT	4.19	0.042*	0.60	0.440	1.53	0.216
AT	21.9	<0.0001*	61.7	<0.0001*	44.0	<0.0001*
LT × PT	0.23	0.638	0.003	0.958	0.31	0.581
LT × AT	1.73	0.190	2.57	0.109	2.06	0.151
PT × AT	0.03	0.851	0.01	0.904	0.02	0.896
LT × PT × AT	1.12	0.255	0.45	0.504	0.89	0.345

LT larval temperature, *PT* pupal temperature, *AT* adult temperature

* Significant difference ($df = 1, 273$)

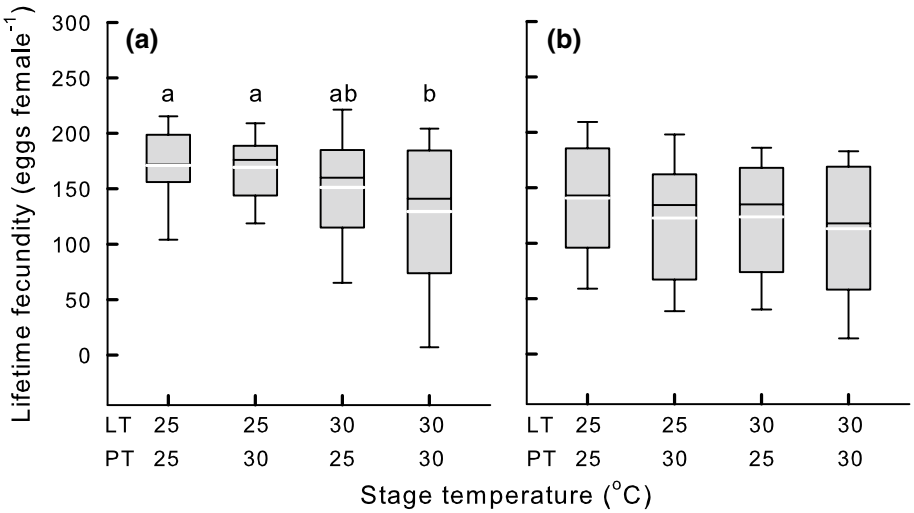

Fig. 4 Box plot for the lifetime fecundity of *P. xylostella* by different larval and pupal temperature treatments. Lifetime fecundity of adults exposed at 25 °C (**a**) or 30 °C (**b**) in different combinations of larval temperature (LT) and pupal temperature (PT). The *upper* and *lower boundary* of the box indicates the 75th percentile and 25th percentile of the data set. The *black* and *white horizontal line* within the box represents the median and mean values. *Error bars* above and below the box indicate the minimum and maximum values. *Different lower-case letters* above each box indicate significant differences ($P < 0.05$) between the four treatments

largely driven by the reduction in female lifespan, but that this was not the case for larval temperatures.

Life-stages differ in their duration. Consequently, in our experiments the number of hot days experienced during the lifetime of an individual inevitably also differed among treatments. For example, when only one of three stages was exposed to 30 °C, adults received on average the most high temperature degree-days (18.9) followed by the larval (13.0) and pupal treatments (7.0). To account for these differences, we tested how lifetime fecundity was related to the total number of hot days using a regression analysis with the sum of the average number of high temperature degree-days experienced in a given life stage as the fixed effect. Overall, fecundity significantly and linearly decreased as high temperature degree-days increased (Fig. 5; $r^2 = 0.11$, $P < 0.0001$).

Duration of oviposition periods

The duration of the oviposition period and post-oviposition period were significantly impacted by the larval or adult treatments (Table 1). Pupal temperature or any interactions between stage treatments were not significant (Table 1). The mean oviposition duration was reduced by high larval temperature and adult temperature by 12 and 32 %, respectively (Fig. 6a). The mean post-oviposition period was increased by 17 % in the high larval temperature treatment or decreased by 37 % in the high adult temperature treatment (Fig. 6b). Both periods non-linearly increased with female longevity, but the significant effect of larval and adult temperatures remained even after accounting for this positive relationship with longevity (ESM Table S1).

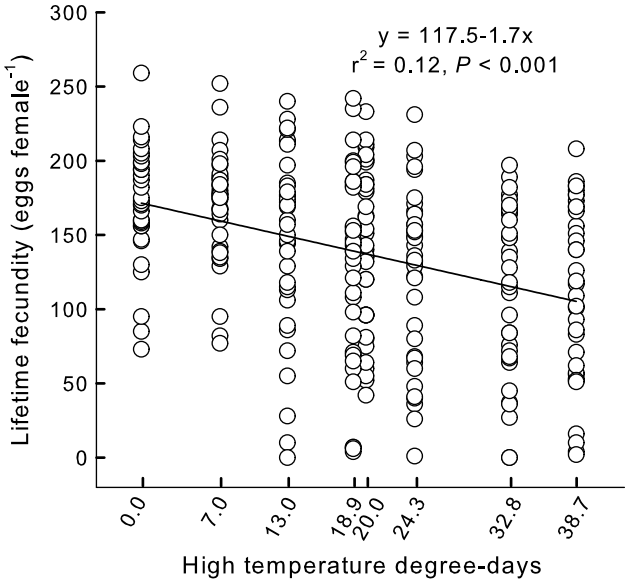

Fig. 5 Relationship between the cumulative number of days above 30 °C which individuals of *P. xylostella* experienced during their life-cycle (high temperature degree-days) and their lifetime fecundity. *0.0*, *7.0*, *13.0*, *18.9*, *20.0*, *24.3*, *32.8*, *38.7* correspond to the larval/pupal/adult temperature treatments at 25/25/25 °C, 25/30/25 °C, 30/25/25 °C, 25/25/30 °C, 30/30/25 °C, 25/30/30 °C, 30/25/30 °C, 30/30/30 °C, respectively

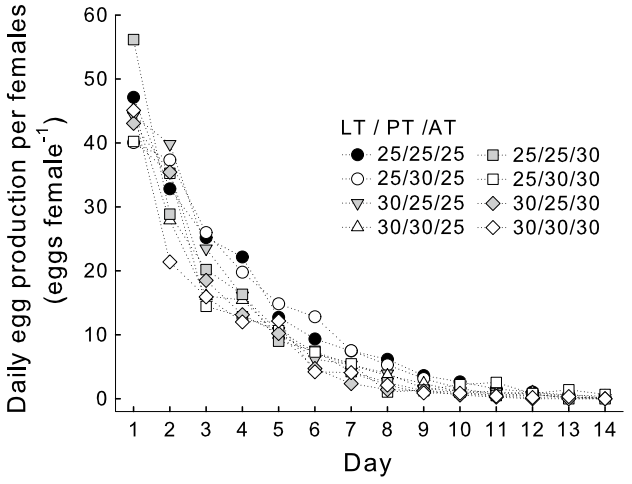

Fig. 7 Mean daily egg production across the oviposition period by different life-cycle stage temperature treatments. Treatments involved combinations of larval temperature (*LT*), pupal temperature (*PT*) and adult temperature (*AT*)

Temporal pattern of daily egg production

Overall, daily egg production exponentially declined with age (Fig. 7), but the temporal patterns of this change were significantly affected by the interaction of the larval and adult temperature treatments and the interaction of the larval and pupal temperature treatments (Table 2). These interaction effects were driven by differences in egg production on the first 2 days and by how rapidly it declined thereafter. In the low temperature treatments during the larval stage, egg production was the highest on the first day (56 eggs) in those treatments when adults experienced high temperatures, but egg production declined much faster at high adult temperatures (49 % reduction) and was always lower than that in the treatments with low adult temperature (35 % decrease) after the second day. In contrast, when larvae experienced high temperature, egg production was on average always higher in treatments with low adult temperature. A similar pattern drove the interaction between larval and pupal temperature treatment. Here, treatments with low pupal temperature always had the highest egg production, with an average of 82 eggs in the first couple of days, but this difference disappeared much faster in the following 4 days in the low larval temperature treatment (with larval/pupal/adult temperatures of 25/25/30 °C) than in high larval temperature treatments (30/25/30 °C or 30/25/25 °C). In general, these

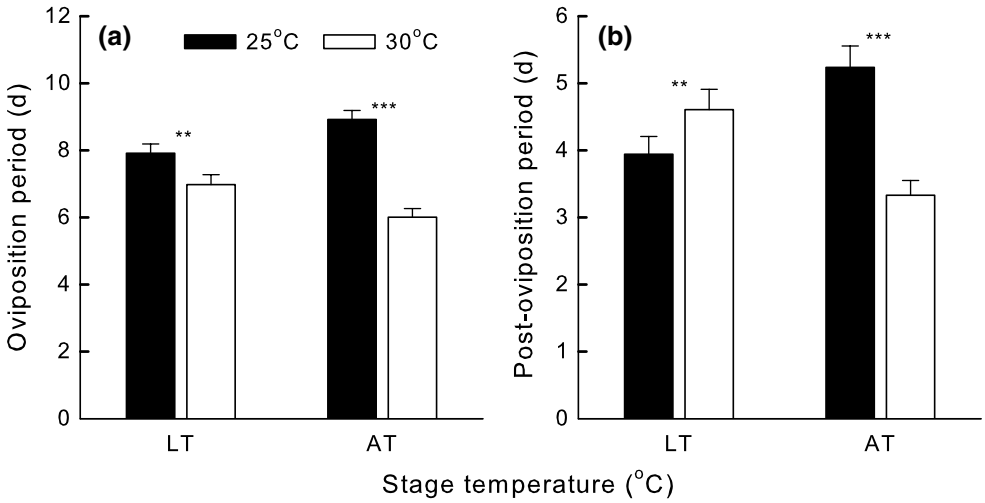

Fig. 6 Mean duration (±SD) of oviposition period and post-oviposition period by larval temperature (*LT*) and adult temperature (*AT*). *Asterisks* indicate significant differences between the larval or adult heat treatments (**$P < 0.01$, ***$P < 0.001$)

Table 2 Treatment effects on temporal patterns of daily egg production by *P. xylostella* at the larval, pupal and adult stages and their interactions

Treatment	*F* value	*P*
LT	$F_{1,272} = 10.4$	0.001*
PT	$F_{1,272} = 3.67$	0.057
AT	$F_{1,272} = 23.4$	<0.0001*
Age	$F_{13,272} = 177$	<0.0001*
LT × PT	$F_{1,272} = 0.12$	0.732
LT × AT	$F_{1,272} = 1.39$	0.239
PT × AT	$F_{1,272} = 0.11$	0.743
LT × Age	$F_{13,272} = 2.80$	0.001*
PT × Age	$F_{13,272} = 2.18$	0.011*
AT × Age	$F_{13,272} = 5.39$	<0.0001*
LT × PT × Age	$F_{13,272} = 3.59$	<0.0001*
LT × AT × Age	$F_{13,272} = 2.17$	0.011*
PT × AT × Age	$F_{13,272} = 1.62$	0.080

* Significant *P* values

significant interactions between temperature treatments and time indicate that the absolute number of eggs produced per day differed among treatments and in how eggs were allocated over the life-span of adults (i.e. proportion of eggs produced on a given day).

Pupal weight

Larval temperature had no significant effect on pupal weight (25 °C: 5.3 mg ± 1.0; 30 °C: 5.5 mg ± 0.8; $t = -0.90$, $P = 0.370$).

Discussion

The frequency and duration of hot periods has increased in recent decades and is expected to continue to increase under global warming (IPCC 2013). Thus, individuals are increasingly likely to experience periods with hot temperatures at multiple stages during their life-cycle. We found that increasing temperatures during some life-stages (but not all) altered adult lifespan, fecundity and oviposition patterns. In addition, changes in temperatures during the early life-stages frequently affected adult oviposition patterns, and these effects were not always additive. Thus, in our study, adults which experienced different temporal patterns of hot periods (i.e. changes in timing and duration) during their life-cycle often had very different demographic rates and oviposition patterns. These results suggest that changes in the timing and duration of hot periods could have strong consequences on the fitness of individuals and population dynamics.

Carry-over effects of high temperatures in organisms with complex (modular) life-cycles

Predicting how periods of high temperatures during early developmental stages influence adult performance is not straightforward in organisms with complex life-cycles. The "lifecycle modularity hypothesis" (Potter et al. 2011) and "adaptive decoupling hypothesis" (Moran 1994; Stoks and Córdoba-Aguilar 2012) both suggest that modular life-cycles may allow invertebrates to assist in dealing with environmental stress. These hypotheses have been supported in laboratory experiments. For example, exposing early life-stages of *P. xylostella* (Xing et al. 2014; Zhang et al. 2015), eggs of *Manduca sexta* (Potter et al. 2011) and *Wyeomyia smithii* (Zani et al. 2005) and early instar nymphs/larvae of *Metopolophium dirhodum* (Ma et al. 2004b) and *Harmonia axyridis* (Knapp and Nedvěd 2013) to acute [i.e. high amplitude but short duration (few hours)] heat stress did not affect female fecundity or adult phenotype. In contrast, in our study, we found that chronic (long term but moderate) high temperatures experienced during the larval and pupal stages reduced almost all aspects of adult performance (e.g. male longevity, female oviposition period, lifetime fecundity) if adults were not exposed to heat stress. Thus, heat stress in the early life-stages clearly carried over to change adult performance, a finding that does not support the "lifecycle modularity hypothesis".

We suggest that the inconsistency between studies which has been observed may be due to potential differences in the types (acute vs. chronic) of heat stress and the life-stage at which the heat stress was imposed, leading to a shift in the balance of injury versus recovery. When the early stages of insects are stressed by a short extreme high temperature, compensatory mechanisms could likely restore all functions during subsequent stages—if individuals do not experience stressful conditions during these stages (Zani et al. 2005; Potter et al. 2011; Xing et al. 2014; Zhang et al. 2015). In contrast, when acute high temperatures applied during late pre-imaginal stages cause severe damage, organisms may not be able to recover before and during the adult stage, resulting in depressed reproduction (Ma et al. 2004b; Zani et al. 2005; Zhang et al. 2015). Consequently, heat stress at a life-stage closer to the adult stage causes more detrimental impacts on adult reproduction (Zhang et al. 2015). Thus, the "life-cycle modularity" hypothesis may only apply to situations with acute heat stress during early stages, while adult performances are often reduced by chronic heat stress, i.e. long-term exposure to moderately high temperatures, such as has been observed in *Drosophila* (Cohet and David 1978), *Stator limbatus* (Stillwell and Fox 2005) and *Neoseiulus californicus* (Nguyen and Amano 2010). Long-term exposure to moderate high temperatures

during the pre-imaginal stage might depress reproduction through a smaller body size (Stillwell and Fox 2005) and decreased number of ovarioles (Cohet and David 1978; Hodin and Riddiford 2000; Bader and Williams 2012). Thus, we would expect that adult performance is negatively correlated with length of the stress period, although differences in heat tolerance across life-stages could muddle this relationship. Consistent with this prediction, we found that adult fecundity was negatively correlated with the total sum (across all life-stages) of hot days which the organisms experienced during their entire life-cycle. These differences in exposure time could also explain why the effect of high temperatures in our experiment was typically smallest when it occurred during the pupal stage, intermediate during the larval stage and highest during the adult stage.

Context-dependent carry-over effects of high temperatures

While our results clearly demonstrate that high temperatures experienced during early life-stages are carried over to affect adult reproduction, these effects were not always additive—rather, they were contingent on the temperature conditions experienced in later life-stages. For example, the effects of exposing larvae to high temperatures on the temporal dynamics of oviposition varied depending on whether pupae or adults experienced moderate or high temperatures. Importantly, this contingency (non-independence) of heat events also emphasizes the importance of the timing of heat events; for example, heat events had a different effect on adult reproduction depending on whether they had affected the pupal stage or adult stage.

Interestingly, we found that the direction of these interaction effects was not always the same, but instead combined effects could be more than additive or less than additive. Post hoc comparisons revealed that although increasing temperature during the pupal stage had on average no significant effects, exposing pupae to high temperatures typically exacerbated the negative effect of high temperatures during the larval stage. In contrast, exposure to high temperatures during the larval or pupal stage frequently did not affect life-time fecundity or longevity if adults also experienced high temperatures. Together, these results clearly indicate that combined heat effects experienced during different life-stages are complex and not independent of each other, emphasizing the importance of the timing of heat events during the life-cycle of organisms.

Stage-specific heat effects and adult reproductive performance

Our results clearly indicate that heat events during the life-cycle can affect adult reproduction but that the effects and underlying mechanisms are stage-specific. The change in life-time fecundity was driven by a complex, stage-specific response to heat. Exposing adults to heat generally reduced fecundity, but we found that this effect was largely indirect and was driven by a reduction in adult life span. In contrast, exposing larvae to high temperatures still significantly reduced fecundity, even when differences in adult life-span were taken into account. It is often suggested that such carry-over effects are mainly driven by changes in growth rate and thus body size of individuals, which in turn affect adult performance (Kingsolver and Huey 2008). However, larval high temperature did not affect pupal mass in our experiments, suggesting that this pattern was not simply mediated by changes in growth rates or mass of adults. This result suggests that heat stress during the larval stage likely has negative effects on egg development, possibly due to a reduction of ovariole number at high temperatures during development (Cohet and David 1978; Hodin and Riddiford 2000; Bader and Williams 2012).

Interestingly, the reduction in life-time fecundity we observed was also associated with a different temporal oviposition pattern, i.e. the proportion and total number of eggs laid per day. Consistent with previous studies, we found that high temperatures generally led to a reduction in maximum egg production at the start of the oviposition period (Krebs and Loeschcke 1994; Janowitz and Fischer 2011; Zhang et al. 2013). However, we also found that high temperatures led on average, to a faster decline in egg production with age (Fig. 7). Together these results emphasize the importance of accounting for complex stage-specific effects of heat events on reproductive performance of organisms. Future studies are needed to identify the specific physiological changes underlying the observed patterns.

Potential application

Understanding how heat events affect the population dynamics of a global pest such as *P. xylostella* is imperative for bio-resource management and pest control in current and future climates. To date, most experimental studies on climate change have investigated how increases in mean temperature affect organisms' performances. However, climate change will also alter the frequency of heat events and when they occur (IPCC 2013). Our results indicate that how heat events affect organisms depends on both the timing and duration of high temperature events. In addition, we found that the effects of successive heat events are often not independent. Taken together, these findings suggest that we cannot predict the effects of current and future climate on natural populations by simply focusing on changes in the mean temperature. Instead, we need to determine when heat events are likely to occur and how long they last to identify which life-stages will be affected. Thus, our

findings emphasize the importance of incorporating the temporal patterns of heat events to describe the dynamics of natural populations and how they will respond to future climate change.

Meanwhile, we have to be careful when we apply the concrete data collected under constant high temperatures. Temporal and spatial thermal heterogeneity is prevalent in nature (Angilletta 2009). The body temperatures of ectotherms are affected by either temporal thermal fluctuations or behavioral thermoregulation by moving from one thermal habitat to another. Consequently, these temperature variations shift the performances of ectotherms in constant environments (Raffel et al. 2013; Vasseur et al. 2014; Colinet et al. 2015). The life performances under constant hot conditions may be weakened by the nighttime recovery (Xing et al. 2014; Zhao et al. 2014) or exacerbated by daytime temperature extremes (Welbergen et al. 2008; Ma et al. 2015a). In addition, ectotherms may also be able to buffer the detrimental impacts of high temperatures through behavioral thermoregulation (Kearney et al. 2009; Ma and Ma 2012a, b).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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