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Fitness under high temperatures is overestimated when daily thermal fluctuation is ignored

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ABSTRACT

Experimental studies on the thermal biology of organisms have become crucial to investigate the impact of climate warming. However, most laboratory studies are carried out under constant temperatures and assume a negligible effect from daily fluctuating temperatures. We tested this assumption on multiple fitness traits of the moth *Spodoptera littoralis*, and a literature review on insects complements this study. Tests on *S. littoralis* focused on its optimal and maximal critical temperatures by comparing constant and daily fluctuating temperatures ($\pm 5^{\circ}\text{C}$) at mean temperatures of 25, 29 and 33°C. The nine fitness parameters investigated were influenced by mean temperature. The overall effect was a maximal multiplication rate at 29°C and a marked decrease under the fluctuating regime at 33°C. Effects of fluctuating temperatures differed between mean temperatures. Developmental and larval survival rates at 33°C were lower under the fluctuating thermal regime than under a constant temperature. Our literature review also illustrates that ignoring daily fluctuations based on constant temperatures commonly leads to overestimate fitness traits at high temperatures. Overlooking the experimental bias associated with constant temperatures minimizes the expected impact of climate warming on fitness traits.

Keywords: fluctuating temperature, Jensen's inequality, metabolic cost, CTmax, insects

1. INTRODUCTION

Most organisms face temperature variations during their lifetime because temperature varies hourly, daily, monthly, seasonally, and yearly in most environments. The importance to consider effects of thermal fluctuation on species is well documented (Colinet et al., 2015; Sinclair et al., 2016; Vasseur et al. 2014). Thermal variation can be particularly high throughout the day compared to other temporal scales (Sheldon and Dillon, 2016). Notwithstanding this well-known reality, most laboratory studies are carried out with daily constant temperatures. It is therefore crucial to check the assumption of the studies that results do not differ between constant and daily fluctuating temperatures (*e.g.*, Colinet et al., 2015; Ma et al., 2015; Sinclair et al., 2016; Stoks et al., 2017) and to investigate the causes that might falsify this assumption. Our study focused on fitness traits. We tested the assumption of no difference between constant and daily fluctuating temperatures on multiple fitness traits of the moth *Spodoptera littoralis* and performed a literature review in insects (Appendix A).

In ectotherms such as insects, the response to temperature of most traits is nonlinear and asymmetric, and declines quickly at temperatures above the optimum (Colinet et al., 2015; Martin and Huey, 2008; Paaijmans et al., 2013; Sinclair et al., 2016; Stoks et al., 2017). The thermal performance curve (TPC) of a fitness trait can be characterised by three tipping points: the optimal temperature (T_{opt}) where the value of a fitness trait is maximal and two critical temperatures where the value of a fitness trait is nil (minimal and maximal temperatures, respectively CT_{min} and CT_{max}), as illustrated in Fig. 1. Diverse definitions of critical values can be found in literature, notably a recent approach to define the upper thermal limit of overall fitness in ectotherms (Jørgensen et al. 2022; Ørsted et al. 2022). Here, we simply consider the case for a given fitness component (*e.g.*, Huey and Stevenson 1979; Sheldon and Dillon 2016). The fitness difference between constant and daily fluctuating temperatures should depend on the mean temperature studied (*e.g.*, close to T_{opt} , CT_{min} or

CTmax) (Colinet et al., 2015; Stoks et al., 2017) as well as on the breadth of variation in fluctuating temperatures (Colinet et al., 2015; Stoks et al., 2017). The combination of the mean temperature and breadth of variation can be summarised by the range of fluctuating temperatures investigated. In our study on *S. littoralis*, we focused on ranges around T_{opt} and CTmax by comparing constant and daily fluctuating temperatures at relatively high temperatures (mean temperatures of 25, 29 and 33°C, with a daily variation of 5°C around each mean temperature). Indeed, insects will be more frequently challenged by high temperatures with climate warming (Kearney et al., 2009). Moreover, the responses of traits are predicted to be particularly sensitive to high temperatures because T_{opt} and CTmax are close in typical TPCs of ectotherms (Colinet et al., 2015; Martin and Huey, 2008; Paaijmans et al., 2013; Stoks et al., 2017). Previous studies showed that T_{opt} and CTmax are close in *S. littoralis* studied under constant temperatures (Bagni et al., 2022; Massot et al., 2021).

Fig. 1 illustrates our predictions for the responses of fitness traits under thermal ranges close to T_{opt} (Fig. 1A) and CTmax (Fig. 1B). Under these thermal ranges, the fitness difference between constant and fluctuating temperatures can have different causes. As a first cause, nonlinear TPCs lead to unbalanced effects between temperatures that fluctuate below and above a mean temperature (Ruel and Ayres, 1999). The overall response of a trait can be predicted from the mathematical property of the form of nonlinear functions known as Jensen's inequality (Ruel and Ayres, 1999). By definition, the function around T_{opt} (where fitness is maximal) is a decelerating function (the convex part of the TPC in Fig. 1). The prediction for a decelerating function is that the response of traits will be worse under fluctuating temperatures than constant temperatures (Colinet et al., 2015; Ruel and Ayres, 1999; Stoks et al., 2017). A second cause of the differential response between fluctuating and constant temperatures is related to metabolism. The energetic cost of metabolism in ectotherms is important in the thermal range of high temperatures because the metabolic rate

tends to increase exponentially as temperature increases (Colinet et al., 2015; Ruel and Ayres, 1999). This accelerating function between metabolism and temperature leads to another effect of Jensen's inequality, with a metabolic cost higher under fluctuating than constant temperatures. We discuss the effect of metabolic cost independently of Jensen's inequality because metabolism is not a fitness trait but may influence fitness traits. A third cause of the differential response between fluctuating and constant temperatures involves the influence of the critical temperature CT_{max} (Ma et al., 2015). Temperatures close to CT_{max} are known to result in detrimental effects related to heat stress. Although heat stress induces the synthesis of heat shock proteins (HSPs) that allow organisms to cope with stronger and longer heat stress, even the production of HSPs has negative effects (Colinet et al., 2015; Feder and Hofmann, 1999). These negative effects can be associated with energetic cost, consumption of nutrients, toxicity at high HSPs concentrations, and detrimental interactions with cellular processes (Feder and Hofmann, 1999). More generally, fluctuating temperatures around CT_{max} result in shifts between permissive temperatures and stressful higher temperatures. Because stressful temperatures beyond CT_{max} cause injuries, fluctuating temperatures around CT_{max} should accumulate injuries or costs to repair injuries (Jørgensen et al. 2022).

We expect additive effects of the three causes of difference between constant and fluctuating temperatures (Jensen's inequality, metabolic cost, CT_{max} closeness) because they involve different processes that are not mutually exclusive, and Fig. 1 synthesizes our predictions. We predict lower fitness values under fluctuating than constant temperatures, both for thermal ranges close to T_{opt} and CT_{max} (respectively, the T_{opt} and T_{high} scenarios in Fig. 1). However, a larger difference in the T_{high} scenario is expected because of a higher metabolic cost and CT_{max} closeness. The difference in metabolic cost between fluctuating and constant temperatures should be higher under the T_{high} than T_{opt} scenario because metabolism usually increases exponentially as temperature increases (Colinet et al., 2015;

Ruel and Ayres, 1999). In summary, we expect lower fitness values in the fluctuating thermal regime than in the constant regime at optimal and high temperatures but with a clearer difference at higher temperatures. Our experimental study that tested this pattern is supplemented with a literature review on fitness traits in insects to synthesize findings on differences of fitness traits between constant and fluctuating temperatures.

2. MATERIALS AND METHODS

2.1. *Study species*

The moth *S. littoralis* is a widespread Mediterranean and African pest (CABI, 2020). This species is well-known, easy to rear in laboratory, with facilities to estimate fitness traits, and several studies have provided key knowledges on its thermal biology. Under constant temperatures, the thermal optimum of the population multiplication rate of *S. littoralis* was estimated at 29 °C in our previous studies (Bagni et al., 2022; Massot et al., 2021). However, the response of this species to temperature differed among studied parameters, thus showing the importance of studying the effects of temperature on multiple parameters (Mordecai et al., 2019; Sinclair et al., 2016). When larval and pupal survival rates responded moderately to temperatures varying from 23 to 29°C, the development of *S. littoralis* dramatically accelerated, particularly between 23 and 27°C. This strong thermal sensitivity of the development rate explains why this species can complete two to seven generations per year depending on regional climatic conditions (Khafagi et al., 2016). Moreover, it was found that adults of *S. littoralis* cannot reproduce at a constant temperature of 30 °C (Sidibé and Laugé, 1977). This value of CTmax for reproduction also seems close to CTmax for pupal survival, which Sidibé and Laugé estimated to be low at 30°C. This response is in contrast to the high

pupal survival rates observed at 29 °C in our previous studies (Bagni et al., 2022; Massot et al., 2021) and provides evidence for the proximity of T_{opt} and CT_{max} .

2.2. Experimental setup

The *S. littoralis* used in the study were obtained from a laboratory strain maintained at 23 °C, 60-70 % relative humidity, and a 16:8 light/dark photoperiod, with larvae fed *ad libitum* with semiartificial food (Hinks and Byers, 1976). We obtained experimental clutches by mating pairs of one female and one male (two-day-old adults) in plastic boxes (10 cm in diameter) with sugar water (20 g/L) as food and a strip of filter paper to more easily collect the clutches. We made 105 mating pairs to obtain 56 clutches. Clutches were collected at the peak of egg laying 24 hours after mating (Kehat and Gordon, 1975). Clutches were divided into two parts to rear them under the two different thermal regimes (constant *versus* fluctuating regimes) of the same mean temperature (25, 29 or 33°C). We used this design to avoid a sampling bias related to clutch effects (Massot et al., 2021) in our comparison between constant and fluctuating regimes. The fluctuating regime was a daily temperature fluctuation of $\pm 5^\circ\text{C}$ (Appendix B). The daily fluctuation of $\pm 5^\circ\text{C}$ is common in natural environments (Wang and Dillon, 2014) and commonly used in studies on fluctuating thermal regimes (Verheyen et al., 2022). Six environmental test chambers (Panasonic MLR-352H) were used in parallel (one per experimental temperature) to control temperatures independent of relative humidity, which was maintained at 70 %.

We performed a daily survey of experimental boxes (225 x 145 x 72 mm) to monitor larvae and pupae until adult emergence. We standardized the density to 30 larvae per experimental box when larvae reached the 4th larval instar. The larval survival rate (hereafter called late larval survival rate) was measured from the 4th larval instar to pupation. Sex was identified at pupation, and pupal survival was quantified. From this survey, we also measured

the developmental rate as $1/\text{developmental duration (days}^{-1}\text{)}$ (Lyons et al., 2013; Paaijmans et al., 2013) between the egg stage and adult emergence. Developmental rate can be used as a fitness proxy for generation time (Huey and Berrigan, 2001). Finally, we estimated the mating rate of two-day-old adults from mating pairs of one female and one male that were exposed to the same temperature treatment (called final mating rate, and computed as the proportion of mating pairs which produce a clutch after 24 hours), as well as the hatching rate of clutches they produced (called final hatching rate). This experiment was performed in February 2020. It was complemented with an experiment in May-June 2022 to quantify the initial hatching rate of eggs at the beginning of the temperature treatments and the early larval survival rate of larvae between the 1st and 4th instars. These parameters were obtained using estimates of the number of eggs we obtained from the clutch mass (Appendix C: $R^2 = 0.95$ for the relationship between the number of eggs and clutch mass). We counted the number of hatched eggs to quantify the hatching rate and the number of larvae that reached the 4th larval instar to quantify the early larval survival rate.

2.3. Data analyses

Appendix D provides the sample sizes for all our variables in each of our six experimental treatments that crossed the two thermal regimes and three mean temperatures. We tested the effects of the thermal regimes, mean temperatures, and their interaction. We analysed all our variables using logistic models. In particular, we discretized our continuous variables (hatching rate, early larval survival rate, developmental rate) that did not satisfy normality or homoscedasticity, even when log transformed. Hatching rate and early larval survival rate were discretized into binomial variables with regard to their median value. We had to discretize the developmental rate with regard to the median values of each mean temperature to maintain variation in analyses. Indeed, the overlap between the developmental

times of mean temperatures was very limited. The 99 % confidence interval of the developmental time for females was 0.0321-0.0324 days⁻¹ at 25 °C, 0.0416-0.0422 days⁻¹ at 29 °C, and 0.0459-0.0474 days⁻¹ at 33 °C, and the 99 % confidence interval of the developmental time for males was 0.0319-0.0323 days⁻¹ at 25 °C, 0.0418-0.0424 days⁻¹ at 29 °C, and 0.0452-0.0464 days⁻¹ at 33 °C. Analyses were carried out with JMP software (JMP Pro 15, SAS Institute Inc., Cary, NC), using stepwise removal of terms with $P > 0.10$.

We estimated the multiplication rate at the population level using demographic models (Caswell, 2001). We parameterised these models with all our fitness estimates: developmental rate, survival rate from 1st to 4th larval instars, survival rate from 4th larval instar to pupation, pupal survival rate, mating rate and hatching rate. In brief, the life cycle of *S. littoralis* was modelled with an age-structured matrix (where each age class was of 1 day) following the methodology detailed in Massot et al. (2021). The age-structured matrix projects how the number of individuals in each age change between successive generations and the asymptotic population growth rate is quantified by the dominant eigenvalue of the matrix (Caswell, 2001). Modelling was achieved with the program ULM ('Unified Life Models') (Ferrière et al., 1996; Legendre and Clobert, 1995). The models corresponding to the six experimental treatments were parameterised with the fitness estimates of the study provided in Appendix E. We performed two sets of models to estimate the multiplication rates considering the initial or final hatching rate of the experiment. Indeed, the thermal sensitivity of the hatching rate of eggs at the beginning of the experiment differed dramatically with the hatching rate of eggs after the full life cycle under the temperature treatments.

2.4. Literature review on fitness traits in insects

We searched the literature using the keywords 'temperature', 'thermal', 'fluctuating', 'variability' and 'variation'. Our selection criteria were for studies on fitness traits in insects

that compared constant and daily fluctuating temperatures with the same mean temperature between the two thermal regimes. In addition, we did not select studies with a marked asymmetrical fluctuation around the mean temperature or studies based on daily alternating temperatures (*i.e.*, with a rapid shift between only two temperatures). Although we observed an increasing number of studies that compared constant and daily fluctuating temperatures during the last decade, we found only 22 studies with our selection criteria (see the references listed in Appendix A). Because thermal sensitivity differs between species (Angilletta, 2009), we defined classes of temperatures with regard to the optimal range of species (Cold, Optimum and Hot classes for values inferior, around or superior to the range of optimal temperatures, respectively).

3. RESULTS

3.1. Comparison of constant and fluctuating temperatures in *Spodoptera littoralis*

Table 1 shows the tests of the effects of mean temperatures (25, 29, and 33°C), thermal regimes (constant *versus* fluctuating regimes), and their interaction. The nine fitness parameters tested were influenced by mean temperature ($P < 0.001$ in all tests). If the developmental rates of females and males increased with temperature (Fig. 2E and F), the seven other fitness parameters decreased at the highest temperature of 33°C (Fig. 2A to D for the four survival rates tested, and Fig. 3A to C for the three reproductive parameters tested). As we expected, these detrimental effects indicated that the developmental temperature of 33°C was close to the CTmax of *S. littoralis*. In addition, the analyses revealed significant interactions between the effects of mean temperature and thermal regime on the female pupal survival rate and the developmental rates of females and males (Table 1). At the highest temperature of 33°C, these fitness parameters were lower under the fluctuating thermal

regime than under the constant temperature regime (Fig. 2C, E and F). Moreover, we found a significant effect of thermal regime on the late larval survival rate (Table 1). However, the late larval survival rate was particularly lower under the fluctuating regime than at constant temperature at the mean temperature of 33°C (Fig. 2B), as supported by the marginally significant interaction between the mean temperature and thermal regime (Table 1) and local tests per mean temperature (thermal regime effect with $P < 0.001$ at 33°C, and $P > 0.10$ at 25 and 29°C).

3.2. Integrative estimate of fitness traits

Our fitness estimates were integrated into demographic models based on the life cycle of *S. littoralis*. We simulated the six experimental scenarios that crossed the three mean experimental temperatures with the two thermal regimes (values used to fix parameters are reported in Appendix E) to obtain their corresponding population multiplication rate. The multiplication rate of the simulated populations was the highest at 29°C, and its decrease at the higher temperature of 33°C was more marked under the fluctuating thermal regime (Fig. 4A). Therefore, the overall impact of the high temperature appeared to be underestimated under the constant thermal regime. This result was obtained from the modelling of the effects of mean temperatures and thermal regimes from the egg stage to adult emergence (using the estimates of the initial hatching rate reported in Fig. 3A). However, multiplication rates are to consider *in fine* as nil at 33°C for both thermal regimes (Fig. 4B) because adults were not able to reproduce after the full life cycle at this temperature (Fig. 3C).

3.3. Review of fitness traits in insects

We reviewed the literature for studies on fitness traits in insects that provide statistical tests comparing constant and daily fluctuating temperatures (see our selection criteria in

Materials and Methods). As an encouraging sign, we observed an increasing number of studies that compared constant and daily fluctuating temperatures during the last decade (Appendix A). Overall, constant and daily fluctuating temperatures commonly differed. Significant differences between temperature regimes were found in 81 out of 145 tests (Fig. 5). These differences were found in 70% of tests on cold temperatures, 46% of tests on temperatures around optimum, and 68% of tests on hot temperatures. The other finding of the review is the reverse patterns between cold and hot temperatures (Fig. 5). The fitness traits observed to be higher in the fluctuating than constant regime were the most frequent in tests of cold temperatures. In contrast, the fitness traits were more frequently lower in the fluctuating regime than in the constant regime in tests of hot temperatures.

4. DISCUSSION

We studied *S. littoralis* at relatively high temperatures of 25, 29 and 33°C, a thermal range around T_{opt} that we found to be close to 29°C with multiplication rate estimates (Fig. 4). The maximal limit of our thermal range was also close to CT_{max} , as highlighted by the steep decrease in the multiplication rate under the fluctuating thermal regime at 33°C when considering fitness components from the egg stage to adult emergence (Fig. 4A). The study species can have a positive population multiplication rate even with a low hatching rate and a low early larval survival as in our experiment because of the low sensitivity of population multiplication rate to these parameters (Massot et al. 2021). In fact, CT_{max} is below 33°C because adults were not able to reproduce after a full life cycle at 33°C (Fig. 3C and 4B). This result is in accordance with the finding of Sidibé and Laugé (1977) on the lack of reproduction of *S. littoralis* at a constant temperature of 30 °C. The narrow thermal range between T_{opt} and CT_{max} is typical in ectotherms (Colinet et al., 2015; Martin and Huey, 2008; Paaijmans et al., 2013; Sinclair et al., 2016; Stoks et al., 2017). Investigating the fitness

responses of ectotherms around this range is crucial because a small temperature variation around the tipping points of the maximal and nil fitness values leads to a severe fitness decline. It is also critical to study this range of high temperatures because heat waves are becoming more frequent and severe with climate change (Fischer and Schär, 2010; Wang and Dillon, 2014), and even a modest climate warming might have a large negative impact on many ectotherms (Jørgensen et al., 2022).

We predicted lower fitness in the fluctuating thermal regime than in the constant regime at optimal and high temperatures, but with a clearer difference at high than optimal temperature because of higher metabolic cost and CT_{max} closeness (Fig. 1). We found a lower fitness in the fluctuating than constant regime at our high temperature of 33°C, providing evidence for the predominant effect of metabolic cost or/and CT_{max} closeness (injuries caused by heat stress, costs to repair injuries or/and costs of HSPs) at high temperature. Indeed, we did not find a difference between fluctuating and constant regimes at the optimal temperature of 29°C. This lack of difference at the optimal temperature seems to provide evidence for the limited effects of Jensen's inequality and metabolic cost (Fig. 1A).

An alternative to the hypothesis of limited effects of Jensen's inequality and metabolic cost around the optimal temperature is that trade-offs between fitness traits and delayed responses of acclimation may influence the effects of fluctuating temperatures. Indeed, the response of a fitness trait can depend on another fitness trait because of energetic or evolutionary trade-offs (Arias et al., 2011; Marshall and Sinclair, 2010), such as trade-offs between survival and reproduction or between early and late survival (Stearns, 1992). The negative relationships between fitness traits associated with these trade-offs might alter the difference between fluctuating and constant thermal regimes. The delayed responses of acclimation could also obscure the effects of fluctuating temperatures because past stressful temperatures can increase the thermal range of organisms (Colinet et al., 2015) or variable

temperatures can decrease the thermal sensitivity of metabolism (Carter and Sheldon, 2020; Williams et al., 2012). This complexity challenges the predictions for temperatures distant from the extreme critical temperatures CT_{min} and CT_{max} where detrimental effects should predominate, and this complexity might explain the diversity of results under experimental temperatures around optimum (Fig. 5).

Our literature review showed that fitness traits commonly differed between constant and daily fluctuating temperatures. Moreover, the review revealed opposite patterns between cold and hot temperatures. Compared to constant regimes, fitness traits were more frequently higher in fluctuating regimes of cold temperatures and lower in fluctuating regimes of hot temperatures (Fig. 5). Therefore, ignoring daily fluctuations in temperature in studies using constant temperatures often leads to underestimations of fitness traits in cold conditions and overestimations of fitness traits in hot conditions. This reverse pattern was predicted by Stoks et al. (2017) based on the influence of Jensen's inequality and critical temperature CT_{max} , and observed in *Drosophila melanogaster* (Bozinovic et al., 2011).

5. CONCLUSIONS

Although there is an increasing number of studies investigating the effects of daily fluctuations in temperature (Colinet et al., 2015; Stoks et al., 2017), most laboratory studies are still carried out under constant temperatures. In the context of climate warming that is expected to be highly threatening for many ectotherms (Jørgensen et al., 2022), the usual practice to ignore daily fluctuations in temperature will often overestimate fitness traits in hot conditions. As a consequence, this bias will lead to underestimations of the expected impact of climate warming on fitness traits. Furthermore, climate warming is associated with an increase in daily thermal fluctuations (Wang and Dillon, 2014) that could more expose organisms to the negative effects of high temperatures (Vasseur et al., 2014).

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CRedit authorship statement

Thibaut Bagni: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft. **David Siaussat:** Conceptualization, Writing - Review & Editing. **Annick Maria:** Methodology, Resources. **Annabelle Fuentes:** Methodology, Resources. **Philippe Couzi:** Methodology, Resources. **Manuel Massot:** Conceptualization, Formal analysis, Writing - Review & Editing, Supervision, Funding acquisition.

SUPPLEMENTARY DATA

Appendix A - Review of the studies that compared fitness traits in insects

Appendix B - Experimental temperatures

Appendix C - Estimation of the number of eggs

Appendix D - Sample sizes

Appendix E - Values used to fix parameters in matrix models

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TABLE 1 Influence of temperature in constant and fluctuating thermal regimes. Tests of the effects of mean temperature (25, 29, and 33°C), experimental thermal regime (constant *versus* fluctuating regimes $\pm 5^\circ\text{C}$), and their interaction. Significant and marginally effects are reported in bold. The tests of the effect of mean temperature tested on female and male developmental rates were not reported because we had to discretize these variables with regard to the median value of each mean temperature (see Materials and Methods where the non-overlapping 99% confidence intervals between mean temperatures are indicated).

	Mean temperature	Temperature fluctuation	Interaction
Initial hatching rate	$X^2_2=46.7$ $P<0.001$	$X^2_1=0.7$ $P=0.417$	$X^2_2=2.0$ $P=0.360$
Early larval survival rate	$X^2_2=26.1$ $P<0.001$	$X^2_1=0.0$ $P=1.000$	$X^2_2=2.8$ $P=0.242$
Late larval survival rate	$X^2_2=439.5$ $P<0.001$	$X^2_1=10.3$ $P=0.001$	$X^2_2=5.1$ $P=0.079$
Female pupal survival rate	$X^2_2=79.6$ $P<0.001$	$X^2_1=0.5$ $P=0.460$	$X^2_2=8.0$ $P=0.018$
Male pupal survival rate	$X^2_2=161.3$ $P<0.001$	$X^2_1=0.6$ $P=0.448$	$X^2_2=0.7$ $P=0.699$
Female developmental rate	Non-overlapping CI_{99%}	$X^2_1=0.1$ $P=0.763$	$X^2_2=60.4$ $P<0.001$
Male developmental rate	Non-overlapping CI_{99%}	$X^2_1=1.7$ $P=0.199$	$X^2_2=96.9$ $P<0.001$
Final mating rate	$X^2_2=61.7$ $P<0.001$	$X^2_1=0.4$ $P=0.522$	$X^2_2=3.3$ $P=0.195$
Final hatching rate	$X^2_2=36.4$ $P<0.001$	$X^2_1=1.4$ $P=0.229$	$X^2_2<0.1$ $P=1.000$

Figure Legends

FIGURE 1. Expected responses of a fitness trait to constant and fluctuating thermal regimes. Based on a theoretical thermal performance curve (TPC) under the constant regime, the expected deviations of the fluctuating regime are indicated by the red arrows associated with the fitness axis. The double red arrow on the temperature axis indicates an example of range of fluctuating temperatures. TPC is characterised by the optimal temperature T_{opt} where the value of the fitness trait is the highest and by critical temperatures CT_{min} and CT_{max} where the value of the fitness trait is nil. **A:** Predictions for temperatures close to T_{opt} , **B:** predictions for high temperatures (T_{high}). Although the fluctuating regime is predicted to be more detrimental than the constant regime in the two scenarios, we expect this negative effect to be higher in the T_{high} than T_{opt} scenario because of a higher metabolic cost and CT_{max} closeness.

FIGURE 2. Influence of mean temperature and thermal regime on survival and development. Thermal regimes are fluctuating (solid lines) and constant (dashed lines) temperatures. **A:** Early larval survival rate between the 1st and 4th larval instars, **B:** late larval survival rate between the 4th larval instar and pupation, **C:** female pupal survival rate, **D:** male pupal survival rate, **E:** developmental rate between egg stage and adult female emergence, **F:** developmental rate between egg stage and adult male emergence. Vertical lines are s.e.m. between clutches.

FIGURE 3. Influence of mean temperature and thermal regime on reproduction. Thermal regimes are fluctuating (solid lines) and constant (dashed lines) temperatures. **A:** Hatching rate of eggs at the beginning of the experiment, **B:** mating rate after the full life cycle under

the temperature treatments, **C**: hatching rate of eggs after the full life cycle under the temperature treatments. Vertical lines are s.e.m. between clutches.

FIGURE 4. The multiplication rate of simulated populations as a function of mean temperature and thermal regime. Thermal regimes are fluctuating (solid lines) and constant (dashed lines) temperatures. **A**: Multiplication rate before adult reproduction, *i.e.*, considering the estimates of the initial hatching rate (Fig. 3A), **B**: Multiplication rate after adult reproduction, *i.e.*, considering the estimates of the final hatching rate (Fig. 3C).

FIGURE 5. Synthesis from studies in insects that compared fitness traits between fluctuating and constant thermal regimes. From the results compiled from literature (Appendix A), the figure shows the percentage of tests between three temperature classes for fitness traits significantly higher in fluctuating regime (tests with $F > C$), non-significant results (tests ns), and fitness traits significantly lower in fluctuating regime (tests with $F < C$). Numbers above the bars represent the number of tests.

FIGURE 1

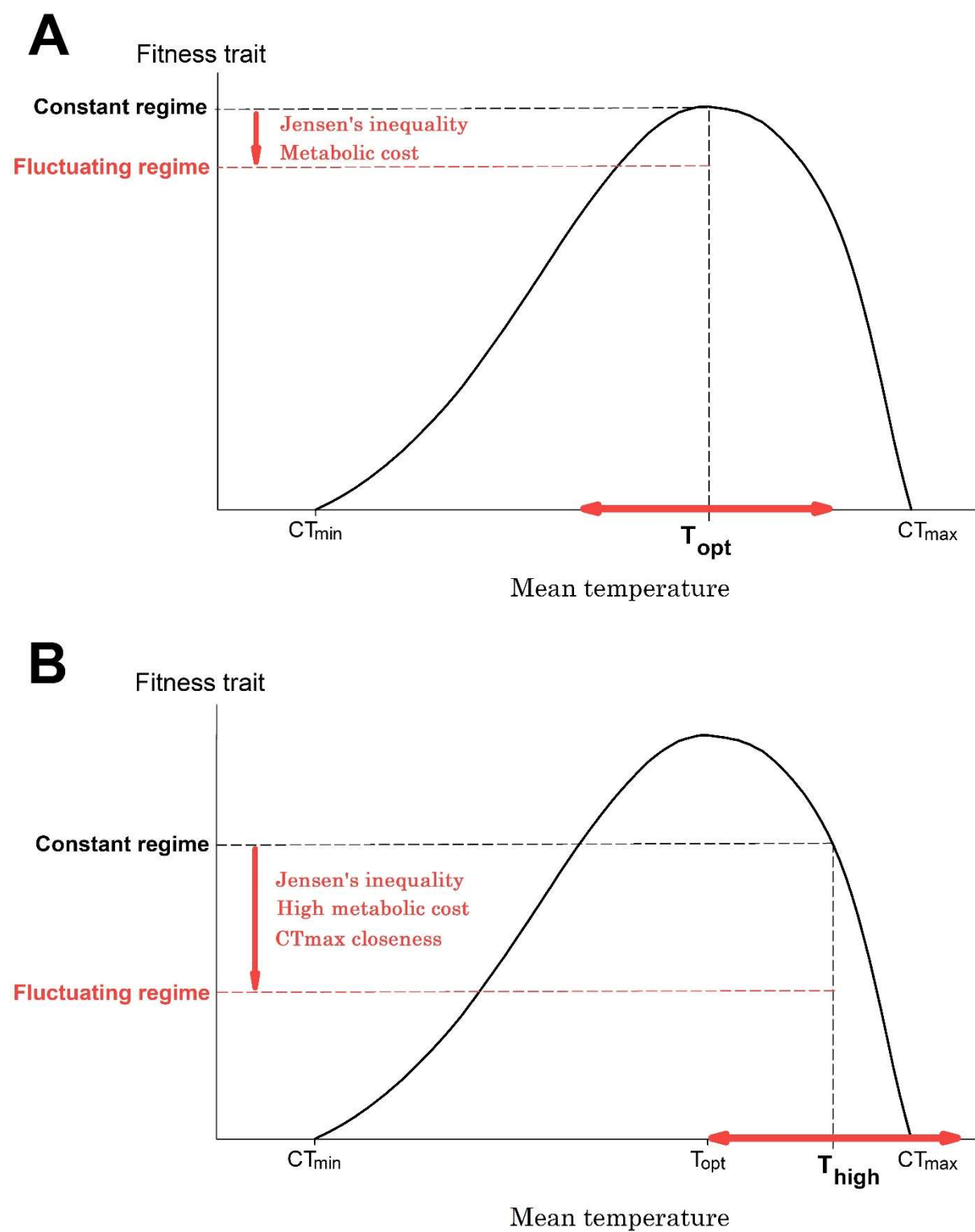


FIGURE 2

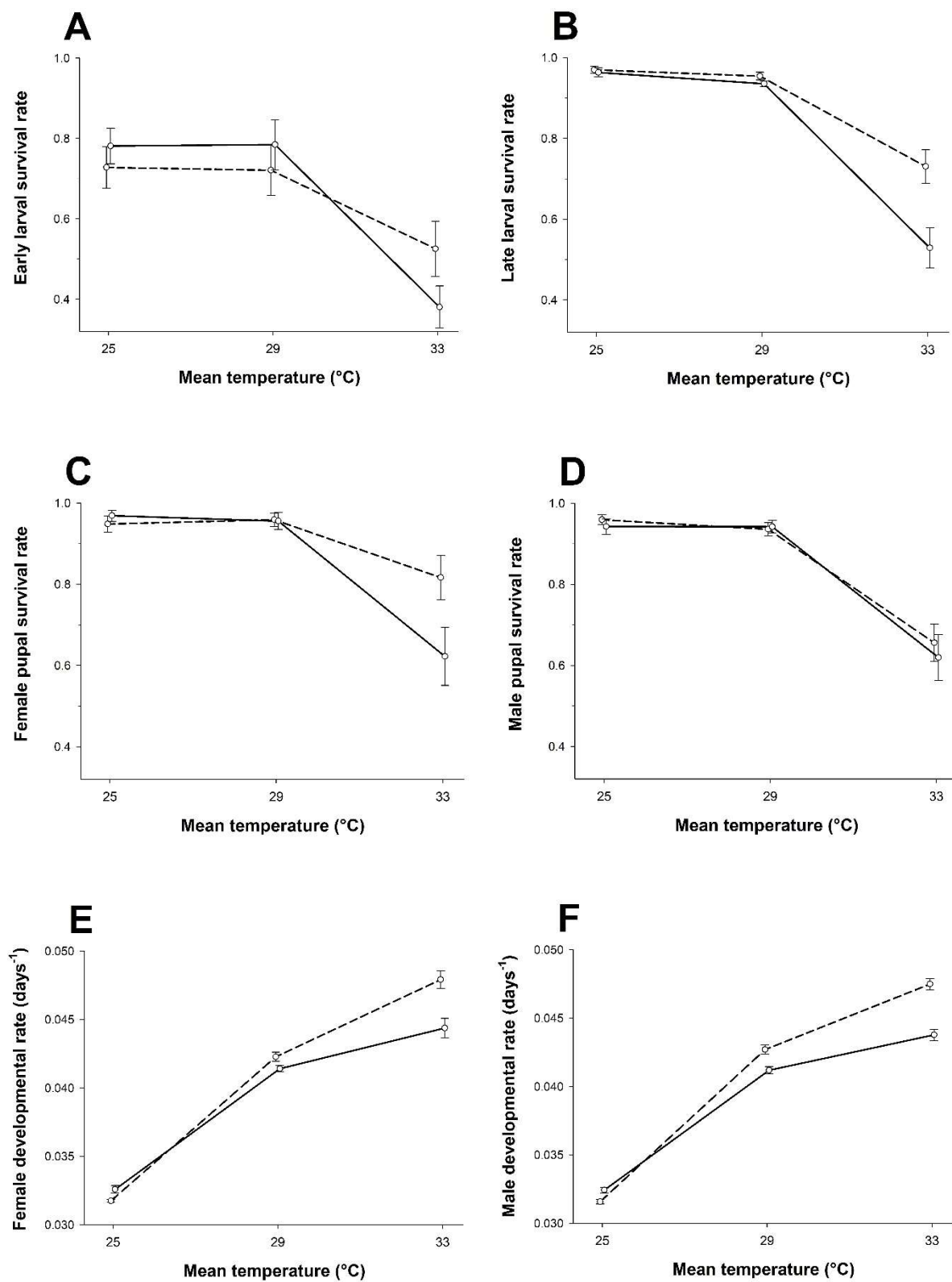


FIGURE 3

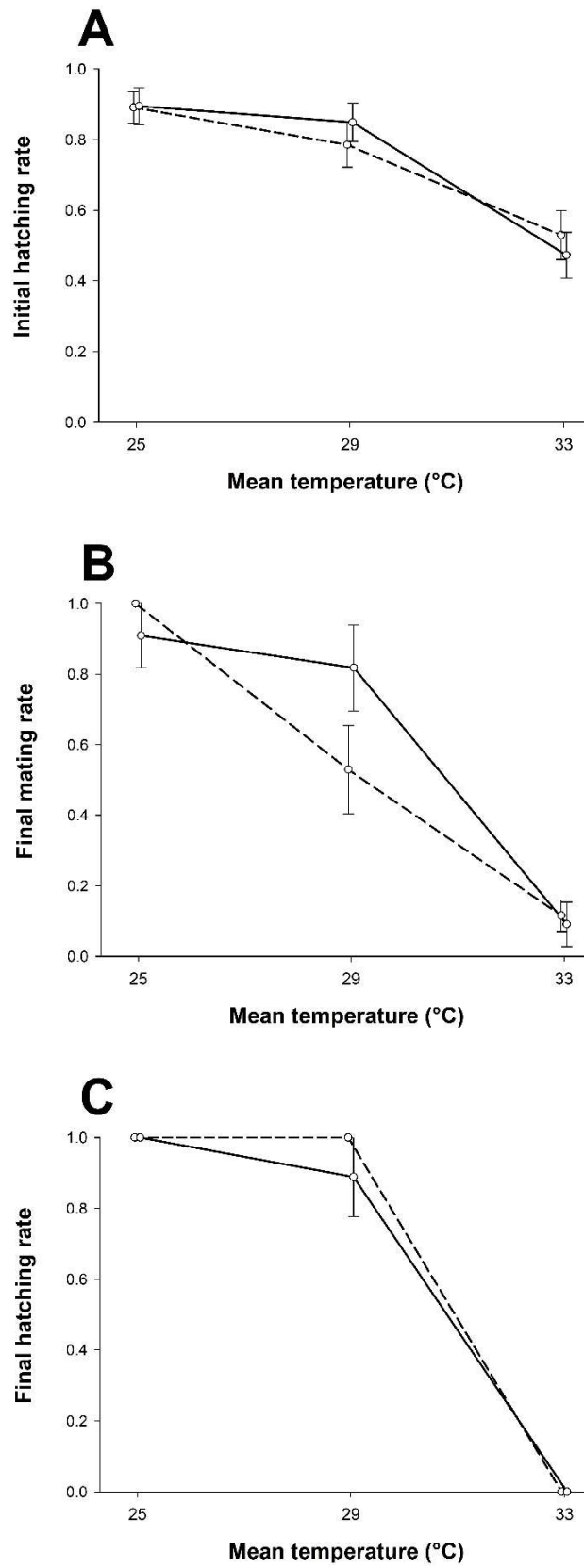


FIGURE 4

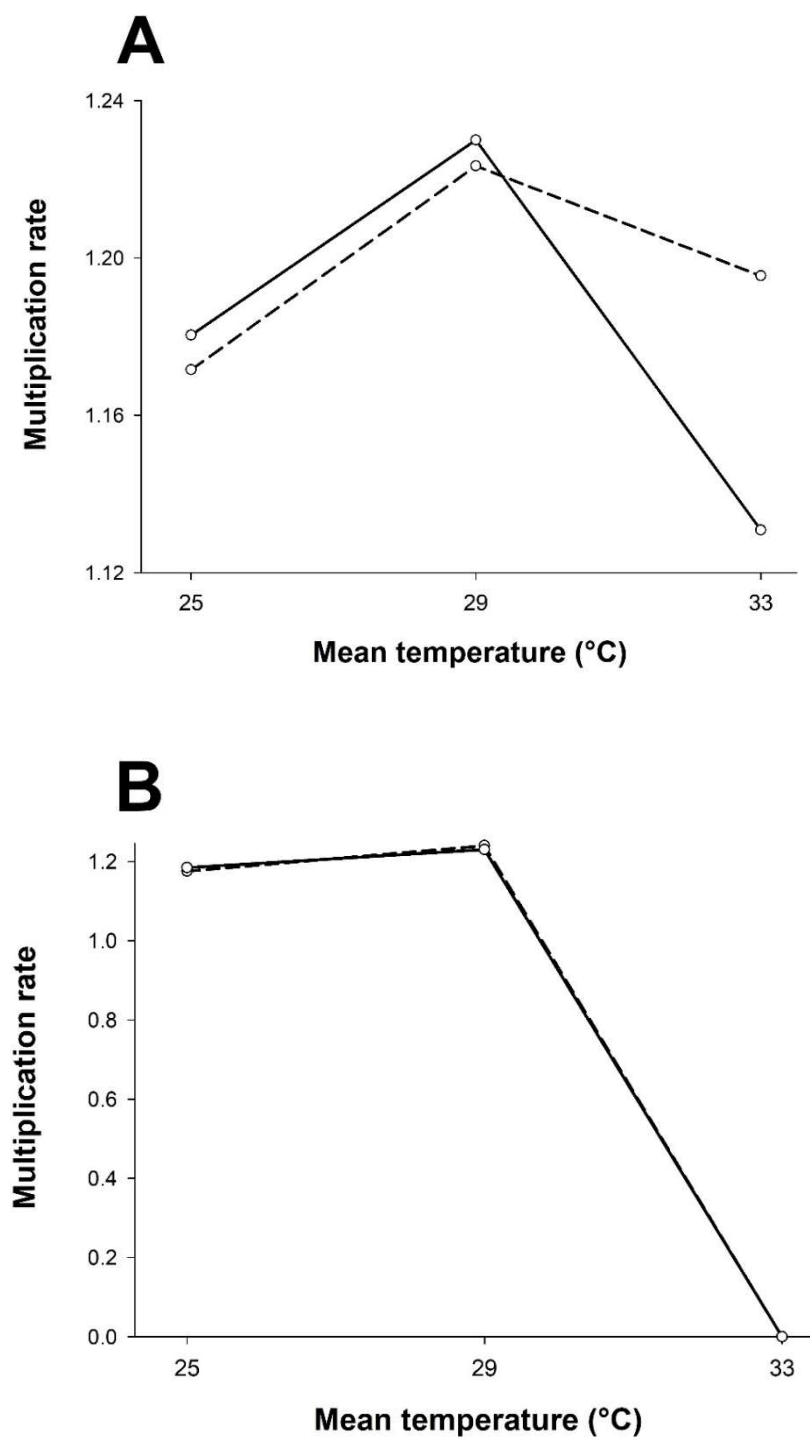
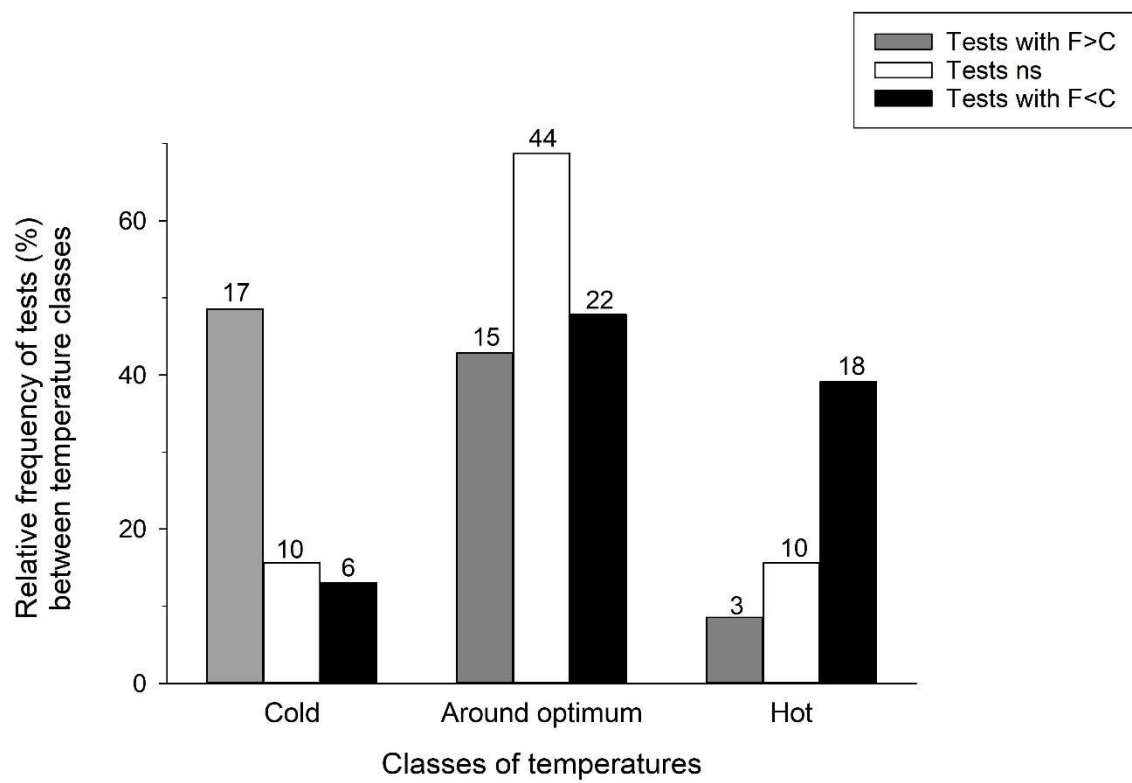


FIGURE 5

APPENDIX A - Review of the studies that compared fitness traits in insects

Table A.1. Review of the studies that compared fitness traits between constant and fluctuating thermal regimes in insects. The results of the comparison between constant (C) and daily fluctuating temperatures (F) are reported according to fitness trait, mean temperature and daily variation studied. Mean temperature is defined by its value and range with regard to the optimal range of species (Cold, Optimum, Hot for values inferior, around or superior to the range of optimal temperatures, respectively). The references (Ref.) are listed below the table, with additional references used to define the range of mean temperatures (Klepsatel et al. 2013 for *Drosophila melanogaster*, Luz et al. 1998 for *Rhodnius prolixus*).

Ref.	Species	Fitness trait	Mean temperature		Daily variation	Result
			Value	Range		
1	<i>Spodoptera littoralis</i>	Larval survival	25°C	Optimum	± 5°C	ns
		Larval survival	29°C	Optimum	± 5°C	ns
		Larval survival	33°C	Hot	± 5°C	F<C
		Pupal survival	25°C	Optimum	± 5°C	ns
		Pupal survival	29°C	Optimum	± 5°C	ns
		Pupal survival	33°C	Hot	± 5°C	F<C in ♀
		Developmental rate	25°C	Optimum	± 5°C	F>C
		Developmental rate	29°C	Optimum	± 5°C	F<C
		Developmental rate	33°C	Hot	± 5°C	F<C
		Reproduction	25°C	Optimum	± 5°C	ns
		Reproduction	29°C	Optimum	± 5°C	ns
		Reproduction	33°C	Hot	± 5°C	ns
2	<i>Grapholita molesta</i>	Developmental rate	25°C	Optimum	± 5°C	F>C

		Larval survival	25 °C	Optimum	± 5°C	F<C
		Pupal survival	25 °C	Optimum	± 5°C	F>C
		Reproduction	25 °C	Optimum	± 5°C	F>C
3	<i>Drosophila melanogaster</i>	Egg to adult survival	20°C	Cold	± 3.5°C	ns
		Egg to adult survival	20°C	Cold	± 5°C	ns
3	<i>Leptopilina boulardi</i>	Developmental rate	20°C	Cold	± 3.5°C	F>C
		Developmental rate	20°C	Cold	± 5°C	F>C
		Success of parasitism	20°C	Cold	± 3.5°C	F>C
		Success of parasitism	20°C	Cold	± 5°C	F>C
4	<i>Culex pipiens</i>	Larval survival	20°C	Optimum	± 3.5°C	ns
		Larval survival	20°C	Optimum	± 7°C	ns
		Adult survival	20°C	Optimum	± 3.5°C	ns
		Adult survival	20°C	Optimum	± 7°C	ns
5	<i>Lycaena tityrus</i>	Developmental rate	18 °C	Cold	± 5°C	F>C
		Developmental rate	24°C	Optimum	± 5°C	F>C
6	<i>Venturia canescens</i>	Fertility	20°C	Cold	± 4°C	F<C
		Fertility	25°C	Optimum	± 4°C	F<C
		Fertility	30°C	Hot	± 4°C	ns
		Adult longevity	20°C	Cold	± 4°C	F>C
		Adult longevity	25°C	Optimum	± 4°C	ns
		Adult longevity	30°C	Hot	± 4°C	F<C
7	<i>Scatophaga stercoraria</i>	Egg to adult survival	18°C	Optimum	± 3°C	ns
		Egg to adult survival	18°C	Optimum	± 6°C	F<C
		Developmental rate	18°C	Optimum	± 3°C	ns

		Developmental rate	18°C	Optimum	± 6°C	F<C
		Adult longevity	18°C	Optimum	± 3°C	ns
		Adult longevity	18°C	Optimum	± 6°C	ns
8	<i>Anopheles arabiensis</i>	Survival	25°C	Optimum	± 5 °C	ns
		Survival	25°C	Optimum	± 10°C	F<C
		Developmental rate	25°C	Optimum	± 5 °C	F>C
		Developmental rate	25°C	Optimum	± 10°C	ns
8	<i>Anopheles funestus</i>	Egg to adult survival	25°C	Optimum	± 5 °C	F<C
		Egg to adult survival	25°C	Optimum	± 10°C	F<C
		Developmental rate	25°C	Optimum	± 5 °C	F<C
		Developmental rate	25°C	Optimum	± 10°C	F<C
9	<i>Drosophila melanogaster</i>	Male mating success	25°C	Optimum	± 4°C	F>C
		Male mating success	29°C	Hot	± 4°C	F<C
		Male fertility	25°C	Optimum	± 4°C	ns
		Male fertility	29°C	Hot	± 4°C	F<C
		Male offspring production	25°C	Optimum	± 4°C	ns
		Male offspring production	29°C	Hot	± 4°C	F<C
10	<i>Wyeomyia smithii</i>	Larval survival	20°C	Optimum	± 8°C	ns
		Larval survival	27°C	Optimum	± 8°C	ns
		Developmental rate	20°C	Optimum	± 8°C	ns
		Developmental rate	27°C	Optimum	± 8°C	F<C
11	<i>Rhodnius prolixus</i>	Survival	24°C	Optimum	± 7°C	F<C

		Fertility	24°C	Optimum	± 7°C	ns
		Fecundity	24°C	Optimum	± 7°C	F<C
		Developmental rate	24°C	Optimum	± 7°C	ns
12	<i>Drosophila birchii</i>	Male reproductive success	17°C	Cold	± 3°C	ns
		Male reproductive success	24°C	Optimum	± 3°C	F<C
13	<i>Cotesia congregata</i>	Survival	25°C	Optimum	± 10°C	F<C
		Survival	28°C	Hot	± 10°C	F<C
		Survival	30°C	Hot	± 10°C	F<C
		Developmental rate	25°C	Optimum	± 10°C	F<C
		Developmental rate	28°C	Hot	± 10°C	F<C
14	<i>Anopheles stephensi</i>	Survival	18°C	Cold	± 4°C	F>C
		Survival	18°C	Cold	± 6°C	F>C
		Survival	26°C	Optimum	± 4°C	ns
		Survival	26°C	Optimum	± 6°C	ns
		Survival	32°C	Hot	± 4°C	F<C
		Survival	32°C	Hot	± 6°C	F<C
		Developmental rate	18°C	Cold	± 4°C	F>C
		Developmental rate	18°C	Cold	± 6°C	F>C
		Developmental rate	26°C	Optimum	± 4°C	ns
		Developmental rate	26°C	Optimum	± 6°C	ns
		Developmental rate	32°C	Hot	± 4°C	F<C
		Developmental rate	32°C	Hot	± 6°C	F<C

15	<i>Chironomus dilutus</i>	Survival	24°C	Optimum	± 5°C	F<C
16	<i>Dysaphis plantaginea</i>	Longevity	12°C	Cold	± 5°C	ns
		Developmental rate	12°C	Cold	± 5°C	F>C
		Fecundity	12°C	Cold	± 5°C	ns
	<i>Aphidius matricariae</i>	Success of parasitism	12°C	Cold	± 5°C	F>C
		Developmental rate	12°C	Cold	± 5°C	F>C
		Longevity	12°C	Cold	± 5°C	ns
17	<i>Protophormia terraenovae</i>	Developmental rate	16°C	Cold	± 12°C	F>C
18	<i>Plutella xylostella</i>	Larval survival	7°C	Cold	± 7°C	ns
		Larval survival	22°C	Optimum	± 7°C	ns
		Larval survival	30°C	Hot	± 7°C	ns
		Pupal survival	22°C	Optimum	± 7°C	ns
		Pupal survival	30°C	Hot	± 7°C	ns
		Adult longevity	7°C	Cold	± 7°C	ns
		Adult longevity	22°C	Optimum	± 7°C	ns
		Adult longevity	30°C	Hot	± 7°C	ns
		Developmental rate	7°C	Cold	± 7°C	F>C
		Developmental rate	22°C	Optimum	± 7°C	ns
		Developmental rate	30°C	Hot	± 7°C	ns
		<i>Diadegma insulare</i>	Success of parasitism	7°C	Cold	± 7°C
	Success of parasitism		22°C	Optimum	± 7°C	ns
	Success of parasitism		30°C	Hot	± 7°C	ns
	Pupal survival		22°C	Optimum	± 7°C	ns

	Pupal survival	30°C	Hot	± 7°C	F<C
	Adult longevity	7°C	Cold	± 7°C	F<C
	Adult longevity	22°C	Optimum	± 7°C	F<C
	Adult longevity	30°C	Hot	± 7°C	ns
	Developmental rate	7°C	Cold	± 7°C	F>C
	Developmental rate	22°C	Optimum	± 7°C	F>C
	Developmental rate	30°C	Hot	± 7°C	F>C
19	<i>Xylotrechus arvicola</i>				
	Egg survival	15°C	Cold	± 3°C	F<C
	Egg survival	18°C	Optimum	± 6°C	F<C
	Egg survival	21°C	Optimum	± 6.5°C	F<C
	Egg survival	30°C	Hot	± 6.5°C	F<C
	Egg developmental rate	15°C	Cold	± 3°C	ns
	Egg developmental rate	18°C	Optimum	± 6°C	F>C
	Egg developmental rate	21°C	Optimum	± 6.5°C	F<C
	Egg developmental rate	30°C	Hot	± 6.5°C	F<C
20	<i>Osmia bicornis</i>				
	Egg survival	17.5°C	Optimum	± 7.5°C	ns
	Egg survival	22.5°C	Optimum	± 7.5°C	ns
	Egg survival	27.5°C	Hot	± 7.5°C	ns
	Larval survival	17.5°C	Optimum	± 7.5°C	ns
	Larval survival	22.5°C	Optimum	± 7.5°C	F<C
	Larval survival	27.5°C	Hot	± 7.5°C	F<C
	Pupal survival	17.5°C	Optimum	± 7.5°C	ns
	Pupal survival	22.5°C	Optimum	± 7.5°C	ns
	Pupal survival	27.5°C	Hot	± 7.5°C	ns
	Adult survival	17.5°C	Optimum	± 7.5°C	F>C

		Adult survival	22.5°C	Optimum	± 7.5°C	F>C
		Adult survival	27.5°C	Hot	± 7.5°C	F>C
		Developmental rate	17.5°C	Optimum	± 7.5°C	F>C
		Developmental rate	22.5°C	Optimum	± 7.5°C	F>C
		Developmental rate	27.5°C	Hot	± 7.5°C	F>C
21	<i>Tribolium castaneum</i>	Fecundity	25°C	Optimum	± 5°C	ns
		Developmental rate	25°C	Optimum	± 5°C	F>C
	<i>Trogoderma inclusum</i>	Fecundity	25°C	Optimum	± 5°C	ns
		Developmental rate	25°C	Optimum	± 5°C	F>C
	<i>Sitophilus oryzae</i>	Fecundity	25°C	Optimum	± 5°C	ns
		Developmental rate	25°C	Optimum	± 5°C	ns
22	<i>Aldrichina grahami</i>	Developmental rate	8°C	Cold	± 3°C	F<C
		Developmental rate	12°C	Cold	± 3°C	F<C
		Developmental rate	16°C	Cold	± 3°C	F<C
23	<i>Drosophila melanogaster</i>	Developmental rate	18°C	Cold	± 4°C	F>C
		Developmental rate	25°C	Optimum	± 4°C	ns

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APPENDIX B - Experimental temperatures

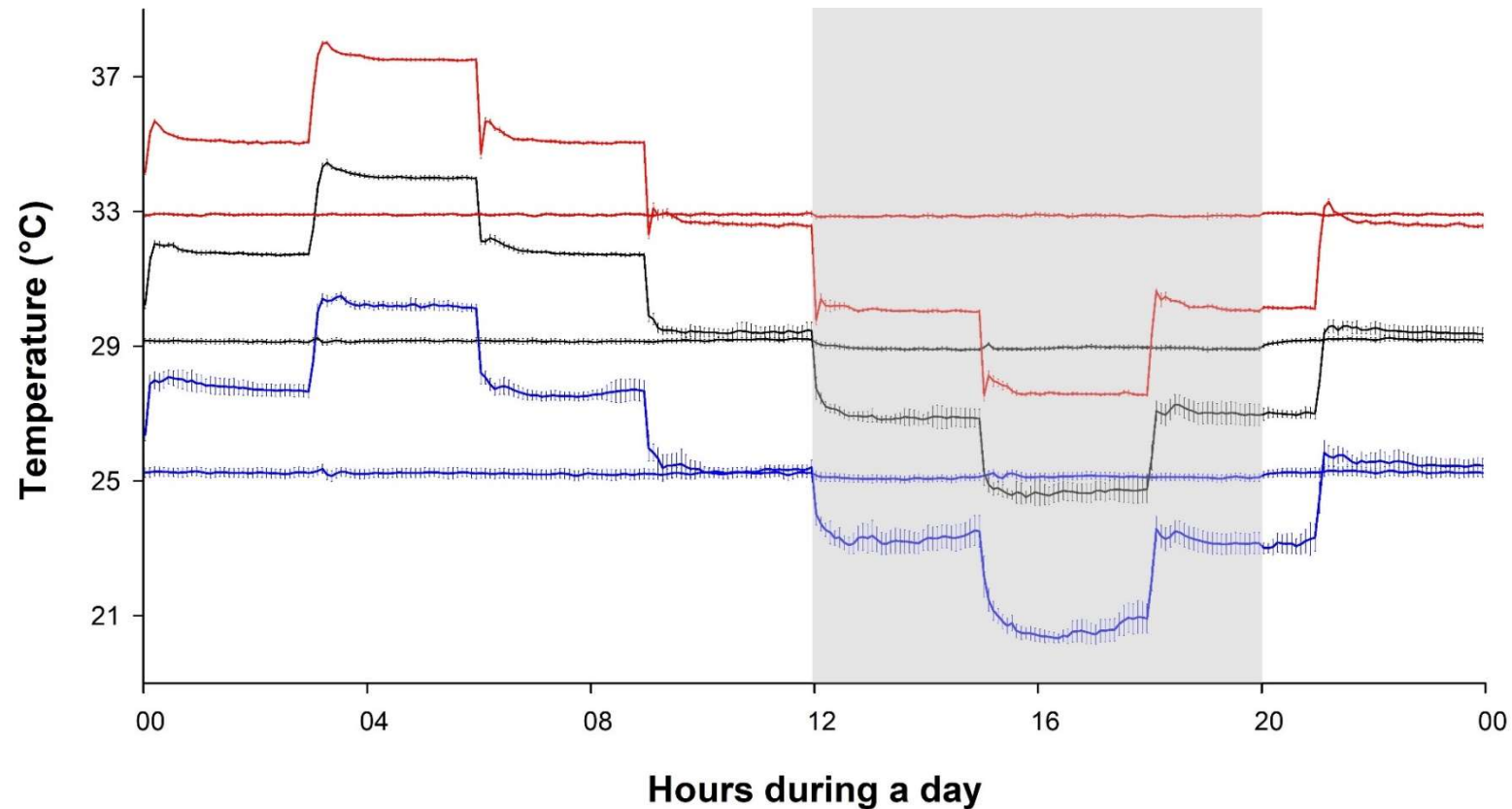


Figure B.1. The six experimental temperatures measured in environmental test chambers. Temperatures were fixed at 25 (blue), 29 (black), and 33°C (red) in constant and fluctuating ($\pm 5^\circ\text{C}$) regimes. Mean values and 95% confidence intervals (vertical bars) were estimated over 24 h of measurements performed each five minutes during three weeks using Vaisala HMP110 Temperature probes. For logistic reasons to study the nocturnal moth *S. littoralis*, the scotophase (grey area of the figure) of the 16/8 light/dark cycle was fixed from 12 to 20h.

APPENDIX C - Estimation of the number of eggs

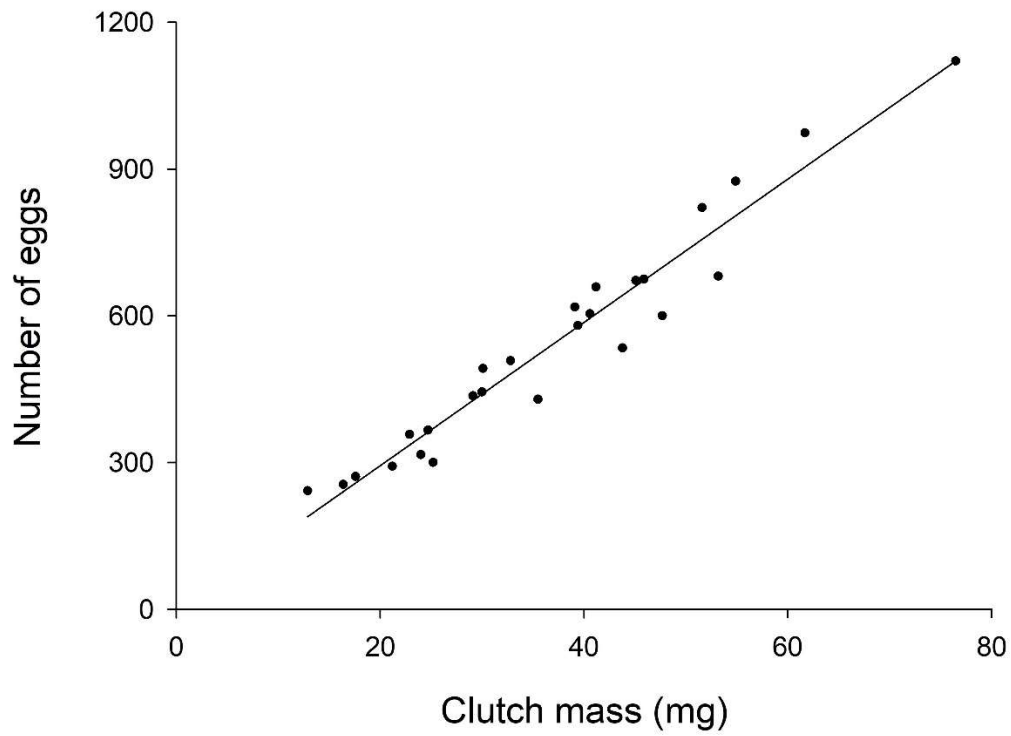


Figure C.1. Relationship between the number of eggs and clutch mass. $R = 0.971$, $N = 26$,
Number of eggs = $0.492 + 14.645 \times \text{Clutch mass}$. The relationship was significant ($P < 0.001$)
and intercept did not significantly differ from 0 ($P = 0.987$).

APPENDIX D - Sample sizes

Table D.1. Sample sizes for the three mean temperatures and two thermal regimes (constant *versus* fluctuating temperatures).

	25°C		29°C		33°C	
	Constant	Fluctuating	Constant	Fluctuating	Constant	Fluctuating
Initial hatching rate ¹	23	23	23	23	24	24
Early larval survival rate ¹	23	23	23	23	24	24
Late larval survival rate ²	600	600	480	510	360	435
Female pupal survival rate ²	251	256	194	224	109	90
Male pupal survival rate ²	271	262	234	225	154	134
Female developmental rate ²	129	145	186	200	85	56
Male developmental rate ²	101	146	219	198	88	83
Mating rate ³	7	11	17	11	52	22
Final hatching rate ¹	7	10	9	9	6	2

¹ Sample sizes are the number of clutches

² Sample sizes are the number of larvae

³ Sample sizes are the number of mating pairs

APPENDIX E - Values used to fix parameters in matrix models

Table E.1. Estimates of the parameters used in matrix models for the three mean temperatures and two thermal regimes. For the parameters indicated with *, we used estimates obtained in Malbert-Colas et al. (2020).

	25°C		29°C		33°C	
	Constant	Fluctuating	Constant	Fluctuating	Constant	Fluctuating
Duration of incubation period (days)	3	3	2	2	2	2
Duration 1 st - 4 th instars (days)	7	6	5	5	4	5
Duration 4 th instar - pupation (days)	10	9	9	9	9	9
Duration of pupal period (days)	12	13	8	8	6	7
Survival from 1 st to 4 th instars (%)	72.8	78.1	72.1	78.5	52.5	38.0
Survival from 4 th instar to pupation (%)	97.0	96.3	95.4	93.5	73.1	52.9
Pupal survival (%)	94.8	96.9	95.9	95.5	81.7	62.2
Sex-ratio (% of females)	48.1	49.4	45.3	49.9	41.4	40.9
Adult female survival until laying (%) *	92.8	92.8	92.8	92.8	92.8	92.8
Laying success after mating (%)	100.0	90.9	52.9	81.8	11.5	9.1
Number of eggs laid *	371	371	371	371	371	371
Initial hatching success (%)	89.1	89.5	78.5	84.9	53.0	47.3
Final hatching success (%)	100.0	100.0	100.0	88.9	0.0	0.0

Reference: Malbert-Colas A., Drozd T., Massot M., Bagni T., Chertemps T., Maria A., Maïbèche M., Siaussat D. 2020. Effects of low concentrations of deltamethrin are dependent on developmental stages and sexes in the pest moth *Spodoptera littoralis*. *Environmental Science and Pollution Research* 27, 41893-901. <https://doi.org/10.1007/s11356-020-10181-9>