



Effects of daily fluctuating temperatures on the *Drosophila–Leptopilina bouvardi* parasitoid association



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ABSTRACT

Koinobiont parasitoid insects, which maintain intimate and long-term relationships with their arthropod hosts, constitute an association of ectothermic organisms that is particularly sensitive to temperature variations. Because temperature shows pronounced natural daily fluctuations, we examined if experiments based on a constant temperature range can mask the real effects of the thermal regime on host-parasitoid interactions. The effects of two fluctuating thermal regimes on several developmental parameters of the *Drosophila* larval parasitoid *Leptopilina bouvardi* were analyzed in this study. Regime 1 included a range of 16–23–16 °C and regime 2 included a range of 16–21–26–21–16 °C (mean temperature 20.1 °C) compared to a 20.1 °C constant temperature. Under an average temperature of 20.1 °C, which corresponds to a cold condition of *L. bouvardi* development, we showed that the success of parasitism is significantly higher under a fluctuating temperature regime than at constant temperature. A fluctuating regime also correlated with a reduced development time of the parasitoids. In contrast, the thermal regime did not affect the ability of *Drosophila* to resist parasitoid infestation. Finally, we demonstrated that daily temperature fluctuation prevented the entry into diapause for this species, which is normally observed at a constant temperature of 21 °C. Overall, the results reveal that constant temperature experiments can produce misleading results, highlighting the need to study the thermal biology of organisms under fluctuating regimes that reflect natural conditions as closely as possible. This is particularly a major issue in host-parasitoid associations, which constitute a good model to understand the effect of climate warming on interacting species.

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1. Introduction

The life-history traits of ectotherms are highly dependent on thermal conditions (Hoffmann et al., 2003; Sinclair et al., 2003; Angilletta, 2009; Steigenga and Fischer, 2009). Physiological processes during insect development are particularly sensitive to temperature variations as are life history traits, such as diapause induction (Beck, 1983), development time (Beck, 1983; Atkinson, 1996), growth rate (Sinclair et al., 2003), body size (Atkinson, 1994), immune function (Karl et al., 2011), and survival and reproduction (Fischer et al., 2003; Marshall and Sinclair, 2010). There have been many experimental analyses of the effects of developmental temperature on a vast number of ectothermic organisms primarily including insect species (e.g., Wieser, 1973; Beck, 1983; Cossins and Bowler, 1987; Sibly and Atkinson, 1994; Atkinson,

1996; Gillooly et al., 2001; Angilletta et al., 2002; Angilletta and Dunham, 2003; Angilletta, 2009). However, organisms in nature must cope with daily and seasonal thermal fluctuations, whereas most experimental data on the consequences of developmental temperature on individual fitness have been obtained in fixed environments, with paradoxically little consideration of daily thermal variation. In addition to accounting for the natural thermal variability, the impact of fluctuating thermal regimes is particularly relevant within the framework of global warming. Ongoing climate change will alter not only the mean temperature but also the variability of thermal conditions, including their daily fluctuation (Tebaldi et al., 2006; Zhou et al., 2009).

The fitness of ectotherms at different temperatures is generally described by a thermal performance curve (Huey and Stevenson, 1979), which rises gradually with temperature from a minimum critical temperature, to an optimum temperature, and then drops rapidly to a critical thermal maximum. For such a nonlinear function, Jensen's inequality constitutes a relevant mathematical tool to predict the effects of environmental variance (Ruel and Ayres, 1999) that will mainly depend on the degree of thermal

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sensitivity of the process (the degree of curvature) and on the amplitude of thermal fluctuations (Martin and Huey, 2008; King-solver et al., 2009; Foray et al., 2014). Jensen's inequality predicts that when the thermal performance curve is concave (in the cold part of curve) the organism should present a higher fitness under constant than fluctuating temperature with the same mean. On the contrary, if the thermal performance curve is convex (the hot part of curve) the organism is expected to have a higher fitness under fluctuating than constant temperature (Pasztor et al., 2000; Foray et al., 2014).

Fluctuating temperatures that remain within the permissive temperature range of the organism generally improve their performance (review in Colinet et al. (2015)). When extended to stressful temperatures, thermal fluctuations can be either beneficial compared to development at constant extreme temperature by allowing a decrease in the effects of extreme temperatures and providing recovery periods from stressful temperatures or by inducing a significant physiological stress that impacts the survival and future reproduction of the organism (Marshall and Sinclair, 2010; Folguera et al., 2011). For instance, Pétavy et al. (2001) showed that by using an alternating regime of 5–15 °C on the two sibling species *Drosophila melanogaster* and *D. simulans*, viability reached about 19%, while an exposition at constant 10 °C was totally lethal. In the same study, they showed that the survival under the alternating regime of 7–29 °C was lower (only 10%) than the viability at constant 18 °C (70–80%). Moreover, Terblanche et al. (2010) showed that a high level of temperature variability dramatically alters the climatic stress resistance for certain traits of the Mediterranean fruit fly (*Ceratitis capitata*), contrary to the results observed with low thermal variance for the same mean temperature.

If there is accumulating evidence of the effects of daily thermal variation compared to constant temperature in ectotherms (see Colinet et al. (2015)), such consequences on multi-specific associations remain less investigated where temperature can influence both directly and indirectly interacting species (but see Blanford et al. (2013)). Parasitoid insects are high trophic level organisms that develop at the expense of other arthropods (usually insects), leading to the death of their host (Godfray, 1994). These parasitoids cause substantial mortality in many phytophagous insect species and, thus, constitute an important component of integrated pest management programs. In this type of association, temperature will impact both the ability of the parasitoid to develop in a host and to circumvent host defense (parasitoid virulence) (Ris et al., 2004), and the ability of the host to defend itself against parasitic attacks (host resistance) (Thomas and Blanford, 2003). In the context of global warming, parasitoids have been the target of numerous studies that showed, by primarily used constant temperature, the crucial role of temperature in mediating the outcome of host-parasitoid interactions (see Terblanche et al. (2015)). For instance, in the silverleaf whitefly (*Bemisia tabaci*) indigenous nymphal parasitoid, *Eretmocerus* sp., the rate of parasitism was higher at warm than at cold temperature (McCutcheon and Simmons, 2001). Moreover, by investigating two lines of the parasitoid *Leptopilina heterotoma* on a different combination of hosts (three *Drosophila* species) and temperatures (4 developmental temperatures), Ris et al. (2004) showed that both host and temperature interacted to shape the parasitoid thermal performance curve. Temperature effect on host-parasitoid interactions is thus direct on the physiology of the parasitoid but also indirect through its host.

Until now, very few studies considered the effects of daily thermal fluctuations on host-parasitoid relationships (Geden, 1997; Sigsgaard, 2000; Liu and Chen, 2001; Blanford et al., 2003; Mironidis and Savopoulou-Soultani, 2009; Reznik et al., 2009; Bahar et al., 2012; Bannerman and Roitberg, 2014) producing

contrasting results. For example, Awadalla (1996) showed that temperature fluctuations during development led to a decrease in the success of the parasitoid *Trissolcus megallocephalus* on the green stink bug (*Nezara viridula*) compared to that observed in a constant thermal regime. In contrast, the emergence rate of the parasitoid *Eumicrosoma blissae* on the oriental chinch bug (*Cavelerius saccharivorus*) increased in fluctuating thermal conditions in comparison to a constant temperature (Sadoyama, 2007).

Leptopilina boulardi (Hymenoptera: Eucolidae) is a specialist parasitoid of *Drosophila* larvae, mainly *D. melanogaster* and *D. simulans* in Western Europe. This parasitoid species has a significant impact on the host population because levels of parasitism (% of parasitized *Drosophila* larvae) can reach 90% in some fruits in southern France (Fleury et al., 2004). *Leptopilina boulardi* is considered as a cold-sensitive species because of its tropical and Mediterranean distribution. In France, the northern limit of its geographical range in 1995 was around the 45°N latitude (Allemand et al., 1999), and recent field sampling has shown that this species has rapidly moved northwards during the last 10 years, possibly in response to global warming (Delava et al., 2014). Experimental studies with constant temperature indicated that *L. boulardi* shows a thermal specialization with an optimum around 25 °C, and its performance falls when the temperature deviates by more than 2 °C from this optimum (Fleury et al., 2009; Moiroux et al., 2012). *Leptopilina boulardi* has been described as an overwintering species with a facultative larval diapause (Claret and Carton, 1980), allowing it to tolerate cold temperatures. Claret and Carton (1980) showed that diapause induction in *L. boulardi* (Guadeloupe strain) was controlled by the temperature, regardless of the photoperiod. Under a constant thermal regime, whereas there was no diapause observed at 25 °C, almost 100% of the larvae entered into diapause at 17.5 °C and the number of diapausing larvae decreased with increasing temperature (14% at 22.5 °C). This thermally induced diapause in *L. boulardi* is unusual and unexpected as it is observed both in temperate and tropical populations (Carton and Claret, 1982). The hypothesis is that the existence of this diapause in the tropical regions should allow the parasitoid to persist in regions of high altitudes and that it could be maintained in the tropical lowlands by gene flow. In temperate regions, the ability of *L. boulardi* to enter diapause should allow it to survive during the long winter period when suitable hosts are rare or absent (Carton and Claret, 1982).

In this study, we compared the effects of fluctuating and constant daily temperatures on several parameters related to diapause and the parasitism success of *L. boulardi*. The temperatures investigated (around 20 °C) are situated on the accelerating convex part of the performance curve of this species, allowing the prediction of a higher performance under fluctuating than under constant temperatures. Our results contribute useful and additional knowledge to better understanding both the consequence of temperature fluctuation and the northward progression of the Mediterranean species *L. boulardi* in a context of high temperature increase in the western continental Europe (Delava et al., 2014).

2. Material and methods

2.1. Parasitoid and host strains

The parasitoids used in this experiment were collected in an orchard located near Valence in southeastern France (latitude 44.58°N), in September 2009 using 12 banana bait traps and ten isofemale lines (lines established by one field-inseminated female) were established. Founding isofemale lines from wild collected females is a widespread technique for investigating the genetic variability of natural populations (see Hoffmann and Parsons

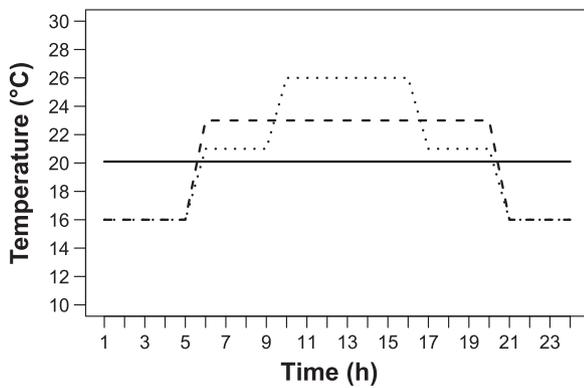


Fig. 1. The three thermal regimes with an average temperature of 20.1 °C used during the development of *Leptopilina boulardi*. The black line represents the constant thermal regime at 20.1 °C, the dashed line represents the fluctuating thermal regime 1 (FTR1) with a phase at 16 °C for 10 h (scotophase) and a phase at 23 °C for 14 h (photophase), and the dotted line represents the fluctuating thermal regime 2 (FTR2) with a phase at 16 °C for 10 h (scotophase), a phase at 26 °C for 5 h 30 min flanked by 2 phases at 21 °C for 4 h 15 min each (photophase).

(1988) and David et al. (2005) for a discussion about the pros and cons of this method). Only one female per trap was used to avoid the collection of genetically related founding females (see David et al. (2005)). These lines were reared on a lab strain of *Drosophila melanogaster* at 25 °C and a photoperiod of LD 14:10 for five generations of full-sib mating before the experiment.

2.2. Thermal regimes

Because one of our aims was to test the effect of temperature fluctuations on diapause induction in *L. boulardi*, we chose three different thermal developmental regimes with a low mean temperature of 20.1 °C and the same photoperiod of LD 14:10 (Fig. 1). This format was chosen based on the results of Claret and Carton (1980), who showed that approximately 50% of *L. boulardi* diapause at 20 °C. In addition to a constant thermal regime at 20.1 °C (constituting the control), we tested a fluctuating thermal regime 1 (FTR1) with 10 h of scotophase at 16 °C and 14 h of photophase at 23 °C (a mean of 20.1 °C) and a fluctuating thermal regime 2 (FTR2) with 10 h of scotophase at 16 °C and 5 h 30 min of photophase at 26 °C flanked by two phases at 21 °C for 4 h 15 min each (a mean of 20.1 °C) (Fig. 1). If a mean temperature of 20.1 °C is close to the lower threshold of the thermal niche of *L. boulardi*, this temperature is within the optimal thermal range of *D. melanogaster*.

2.3. Experimental design

For each thermal regime, we set up 30 vials (10 isofemale lines and 3 replicates per line) with 125 *Drosophila* eggs at 25 °C. After allowing eggs to hatch for 24 h, a single parasitoid female was introduced into each vial for 24 h. To assess the quality of *Drosophila* development in the absence of parasitoids (control vials), we seeded five control vials without *L. boulardi* but with the same number of *Drosophila* eggs for each thermal regime.

Throughout the duration of the experiment (from *Drosophila* eggs until the emergence of hosts and parasitoids), vials were placed in one of the three climate chambers (SANYO, type MLR-351H; temperature fluctuation: ± 0.3 °C), according to the three thermal regimes (35 vials per incubator) under a LD 14:10 photoperiod and 70% relative humidity. The temperature and humidity inside the climate chambers was also recorded and controlled *a posteriori* by a temperature data logger (Lascar, type EasyLog-USB-2) (data not shown). The emergence of adults was checked daily and *Drosophila* and *L. boulardi* individuals were

counted. Adult *Drosophila* that developed in the presence of a parasitoid were subsequently dissected under a microscope by crushing the entire individual between two glass slides, to count the number of flies containing capsules produced by the immune reaction. After 7 days without parasitoid emergence, vials were placed at a constant temperature of 26 °C with a photoperiod of LD 14:10, to end the diapause and complete parasitoid development of diapausing individuals.

2.4. Traits measured

For each thermal regime, we estimated:

- the mean number of adult *Drosophila* emerging from control vials: n_t
- the number of emerging *Drosophila* (n_d) and the number of parasitoids (n_p) counted daily in each vial

We then calculated the survival rate(s) of flies emerging from control vials as $s = n_t/125$, and four parameters of the host-parasitoid relationship (e.g. Chabert et al., 2012):

- Infestation rate (IR), which measures the proportion of parasitized hosts, irrespective of the outcome of parasitism (encapsulation, parasite death during development inducing host death or parasitoid emergence). The IR was calculated as $IR = (n_t - n_d)/n_t$.
- Success of parasitism (SP), which measures the proportion of emerging adult parasitoids among the estimated parasitized host, was calculated for the offspring of each female as follows: $SP = n_p/(n_t - n_d)$.
- The rate of parasite encapsulation (ER), which measures the proportion of parasitoid larvae successfully encapsulated by the host immune response: $ER = n_c/(n_t - n_d)$, where n_c is the number of encapsulated parasitoid eggs in each vial.
- The development time of *L. boulardi* for the three thermal regimes was calculated, starting from the *Drosophila* egg stage by recording daily parasitoid emergence until the last parasitoids emerged.

Then, we calculated the diapause rate of *L. boulardi*, which represents the percentage of parasitoids that entered into diapause. This parameter was calculated for each thermal regime as the ratio between the number of parasitoids emerging after being placed at 26 °C to break diapause and the total number of emerged parasitoids.

2.5. Statistical analysis

The effects of thermal fluctuations on the survival rate of *Drosophila* were analyzed on control vials of each thermal regime using a linear model with the thermal regimes as explanatory variables (qualitative). Despite the very low level of *Drosophila* immune response observed in parasitized vials, we tested if the ER depends on the thermal regime by applying a generalized linear model using a quasipoisson distribution to take overdispersion into account. The development time of parasitoids, IR and SP were analyzed by performing linear mixed models using the *lme* function of the *nlme* package (Pinheiro and Bates, 2000) with thermal regimes as an explanatory variable (qualitative) and line as a random effect. To reach assumptions of normality and homoscedasticity, data were log-transformed for development time, arcsine-transformed for IR and arcsine square-root-transformed for SP. For IR and SP, three experimental vials in which the parasitoid females did not lay any eggs were excluded from the analysis. No statistical analysis was performed on the diapause rate

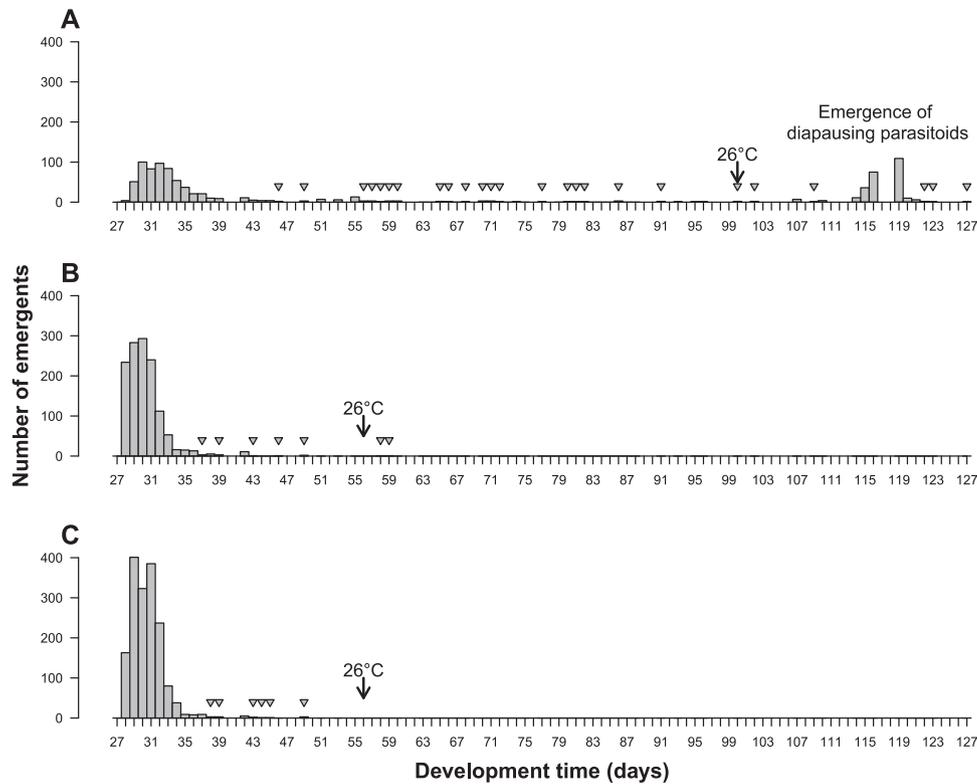


Fig. 2. Effect of thermal regime on the development time and diapause induction of *Leptopilina boulardi*: (A) parasitoids developed at constant temperature, (B) parasitoids developed under fluctuating regime 1 (FTR1) and (C) parasitoids developed under fluctuating regime 2 (FTR2). Arrows indicate the days where parasitoids were transferred to 26 °C to eventually end the diapause and complete their development. Triangles indicate the emergence of one to three parasitoids that are hardly distinguishable in Fig. because of the scale.

because only parasitoids tested in a constant thermal regime entered into diapause. All analyses were conducted with the R studio software (R Development Core Team, 2014).

3. Results

3.1. Survival rate of *Drosophila* hosts without parasitoids according to the three thermal regimes

We found that irrespective of the thermal regime tested, the larval survival rate of *Drosophila* not exposed to parasitism remained unchanged ($s=0.67$ at constant temperature and for FTR2 and $s=0.65$ for FTR1; $df=1$, $F=0.0008$, $P=0.98$).

3.2. Diapause of *L. boulardi*

At a constant temperature of 20.1 °C, a first peak of emergence was observed on the 29th day and it lasted approximately ten days (Fig. 2). Approximately 571 parasitoid adults (over 930 parasitoids in total, i.e. 61.4%) emerged during this first peak. Subsequent emergences occurred sporadically over the following days and then ceased. Approximately two weeks after vials were transferred to a constant temperature of 26 °C, a second lower peak of emergence was observed (~250/930 parasitoids, i.e. 26.9%). This second peak lasted about 8 days and then emergence ceased. We estimated the diapause rate to be about 29% when parasitoids developed at a constant temperature of 20.1 °C.

In contrast, the dynamic of parasitoid emergence was very different under fluctuating thermal regimes, with only one peak of emergence and no diapause in both cases tested (Fig. 2). Insects began to emerge on the 28th day, forming a peak of emergence that reached 1287 in the FTR1 and 1671 individuals, in the FTR2.

The duration of emergence did not exceed 7 days, except for very few emergences that occurred during subsequent days. No parasitoid emergence was observed after the 49th day. Parasitoid emergence did not occur when vials were transferred to 26 °C, suggesting that individuals did not enter into diapause in fluctuating thermal regimes. Finally, we observed no significant difference between either fluctuating thermal regimes (which both suppress the diapause observed at the same mean temperature) but under a constant temperature regime.

3.3. Development time of *L. boulardi*

Since a proportion of parasitoids did not enter diapause under constant temperature, we were able to compare the development time of nondiapausing individuals for the three thermal regimes. The development time was approximately 6 days longer in a constant thermal regime than under fluctuating temperatures (Fig. 3). Fluctuating thermal regimes shorten parasitoid development from 36.27 days at constant 20.1 °C to 30.51 and 30.52 days in fluctuating thermal regimes 1 and 2, respectively; $F=53.45$, $P<0.001$ (Table 1). No significant difference was observed between the two fluctuating thermal regimes.

3.4. Immune response to parasitism, infestation rate and success of parasitism

The rate of parasite encapsulation by *Drosophila* hosts subjected to parasitism for 24 h (ER) was also similar between the three thermal regimes and ranged on average from 0.66% at constant temperature, to 3.05% for FTR1 ($df=2$, $dev=4.88$, $P=0.13$), which is relatively negligible.

There was no significant difference in IR between the three thermal regimes ($F=1.61$, $P=0.21$) (Table 1). On average, the IR

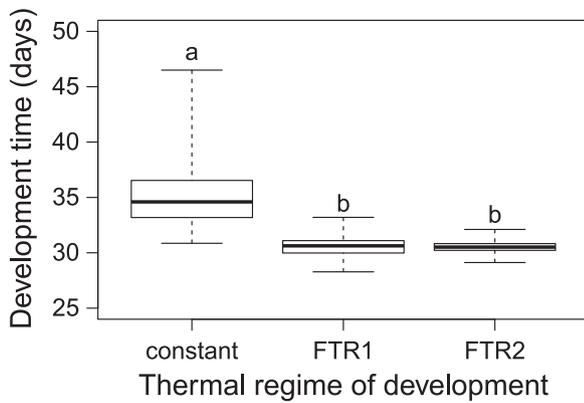


Fig. 3. Effect of developmental thermal regime on the development time of *Leptopilina boulardi*. The boxes range from 0.25 to 0.75 quartile. The thick line inside the box represents the median and whiskers extend from the minimum to the maximum value. Different letters indicate significant differences of development time between thermal regimes.

was 0.65 ± 0.18 , which is slightly less than the values generally observed in this species at a constant temperature of 25 °C (Carton and Frey, 1984; Carton et al., 1987; Boulétreau et al., 1994). The thermal regime did not have any significant effect on the ability of parasitoid females to infest *Drosophila* eggs.

Although the IR was similar between thermal regimes, there were significant differences in the SP between the three thermal regimes ($F=31.25$, $P<0.001$; Table 1). As expected, at constant temperature, the SP was significantly lower than for the two fluctuating thermal regimes, with a mean of 0.40 ± 0.13 (Fig. 4). The SP was also significantly different between the two fluctuating thermal regimes and reached 0.60 ± 0.15 for fluctuating regime 1 and 0.69 ± 0.11 for fluctuating regime 2, suggesting that the peak at 26 °C experienced in the second fluctuating regime significantly improved the SP.

4. Discussion

Our study provides novel data on the effects of fluctuating temperature on host-parasitoid interactions. Our results show that under the same average thermal conditions, a small daily fluctuation in the developmental temperature can have no effect on

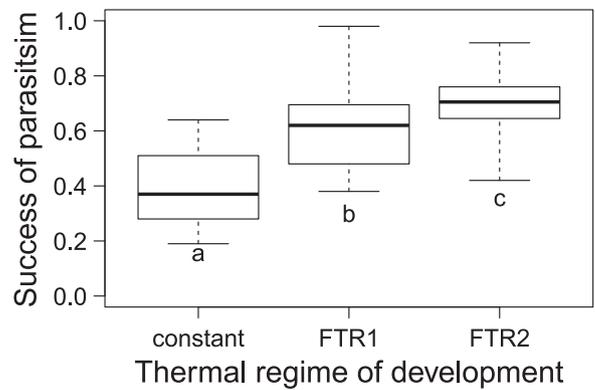


Fig. 4. Effect of developmental thermal regime on the success of parasitism of *Leptopilina boulardi*. The boxes range from 0.25 to 0.75 quartile. The thick line inside the box represents the median and whiskers extend from the minimum to the maximum value. Different letters indicate significant differences of success of parasitism between thermal regimes.

the development of the host but a significant impact on parasitoid success and fitness.

4.1. Impact of temperature fluctuations on the life history traits of *L. boulardi*

4.1.1. Diapause

According to Claret and Carton (1980), we expected a rate of diapause close to 50% in this same species at a constant temperature of 20 °C; however, a rate of approximately 27% at the same temperature was observed. The populations used in these two studies (Guadeloupe vs. southeastern France, sampled in 1980 and 2009, respectively) are very different suggesting that local adaptation might account for the difference in diapause rates. Because diapause is a persistent state even when favorable conditions are transiently reached (Hance et al., 2007), we wonder whether the arrest of development experienced by *L. boulardi* at temperatures below 23 °C is a real diapause. Indeed, the parasitoid is able to break dormancy as soon as the temperature increases again, about 14 days later, in a process that rather is similar to quiescence. Regardless of its name, the arrest of the development of *L. boulardi* at low temperatures restricts its development to a narrow constant temperature range, precluding the detailed

Table 1

Analysis of fixed effects in development time, infestation rate and success of parasitism of *Leptopilina boulardi* in relation to thermal regime. Significant p-values are given in bold. Means and standard error of these three variables are shown for the three thermal regimes.

	Thermal regime			n	Test	d.f.	P
	Constant	FTR1	FTR2				
Development time (days)	35.64 ± 0.76	30.51 ± 0.17	30.52 ± 0.12	84	$F=53.45$	2	< 0.001
Infestation rate (%)	70 ± 0.03	61.25 ± 0.04	63.17 ± 0.03	84	$F=1.61$	2	0.207
Success of parasitism (%)	39.84 ± 0.03	60.19 ± 0.03	69.21 ± 0.02	81	$F=31.25$	2	< 0.001

	Thermal regime			Test	d.f.	P
	Constant	FTR 1	FTR 2			
<i>Drosophila melanogaster</i>						
Survival (%)	67 ± 11.40	65.2 ± 13.18	66.8 ± 9.96	$F=0.0008$	1	0.977
<i>Leptopilina boulardi</i>						
Development time (days)	36.27 ± 4.82	30.51 ± 0.89	30.52 ± 0.64	$F=53.45$	2	< 0.001
Infestation rate (%)	70 ± 14.4	61.25 ± 19.84	63.17 ± 17.78	$F=1.61$	2	0.207
Success of parasitism (%)	39.84 ± 13.30	60.19 ± 15.35	69.21 ± 11.33	$F=31.25$	2	< 0.001

knowledge of the performance curves shape of this species.

Under the same mean temperature, a small temperature fluctuation was sufficient to prevent the entry of *L. boulandi* into diapause. It is known that thermoperiods can alter the induction of diapause, especially in species whose diapause is not dependent on the photoperiod (Beck, 1983). Our results are consistent with those of Musolin et al. (2001), Volkovich and Blumental (1997) and Mironidis and Savopoulou-Soultani (2012), who reported that fluctuating temperatures prevented diapause compared to constant temperatures in *Helicoverpa armigera* and in some species of lacewings, when the photoperiod is ignored. Our results, thus, suggest that warmer thermal peaks during development can extend the thermal niche of this parasitoid.

4.1.2. Development time

We observed a shorter developmental period under fluctuating rather than constant thermal regimes. Using constant temperatures, it is well known that the development time of ectotherms decreases when the temperature increases, up to an optimum temperature (Ratte, 1984; Atkinson, 1996). However, thermoperiods can have profound effects on the development time of insects in comparison to constant temperatures (reviewed in Colinet et al. (2015)), with an accelerating effect of fluctuating thermal regimes in many cases (Beck, 1983; Ratte, 1984; Fischer et al., 2011; Ragland and Kingsolver, 2008; Kingsolver et al., 2009; Radmacher and Strohm, 2011; Bahar et al., 2012; Kjaersgaard et al., 2013). In our study, we used a thermal fluctuation around a mean temperature of 20 °C, which is much colder than the optimal temperature for this species (around 25 °C) (Fleury et al., 2009; Moiroux et al., 2012), and thus is situated in the convex part of its performance curve. Our results (i.e., a significant reduction in the development time of 3 days on average between constant and fluctuating temperatures) under a fluctuating thermal regime are thus consistent with the predictions that can be made following Jensen's inequality (Schoolfield et al., 1981; Niehaus et al., 2012; Foray et al., 2014).

4.1.3. Infestation rate and success of parasitism

The IR did not differ significantly among the thermal regimes tested suggesting that the ability of female parasitoids to infest the host is not affected by the temperature fluctuations used in this study. We found very few studies that compared the effects of infestation rate between constant and fluctuating thermal regimes on host-parasitoid interaction. In our study, the absence of influence of fluctuating thermal regimes on the IR could be related to the fact that *L. boulandi* females show a daily rhythm of parasitization with a main activity just after the end of the light period (Fleury et al., 2000, 2009); a period where the three modalities have almost the same temperature (constant temperature = 20.1 °C, FTR1 = 23 °C and FTR2 = 21 °C; Fig. 1).

In contrast, the ability of parasitoids to develop in *Drosophila* hosts (SP) varies significantly according to the thermal regime tested, with a lower SP at constant temperature than in the two fluctuating thermal regimes (Fig. 4). Since the IR is not affected by the thermal regime, the differences observed in SP might be due to a strong mortality in parasitoids that enter diapause. The SP observed under FTR2 (69%, regime with a peak of temperature at 26 °C for 5 h 30 min) was the highest and is similar to the value observed by Varaldi et al. (2005) (which can reach approximately 70%) at the constant temperature of 25 °C and is close to the optimal temperature of this species (26 °C, Moiroux et al., 2012). Again, this result is consistent with the predictions from Jensen's inequality.

4.2. Consequences on the *Drosophila*-parasitoid community

4.2.1. Northern range expansion of *L. boulandi*

In the south-east of France, *L. boulandi* is currently moving rapidly northwards (about 90 km per decade) concurrently with the increase in both the average temperature and the temperature variation (maximum temperatures increase more than minimum temperatures), especially in autumn (Delava et al., 2014). Since a small fluctuation of the temperature around 20 °C is sufficient to prevent the entry of *L. boulandi* into diapause, autumn can be expected to correlate with a delay of *L. boulandi* into diapause in the context of global warming. This extension of the breeding season could promote the expansion of the parasitoid range into the north of France.

4.2.2. Impact on host-parasitoid relationships

The outcome of host-parasitoid interaction depends on how temperature affects the virulence of the parasitoid versus the resistance level of the host (Thomas and Blanford, 2003). In our study, we showed that the thermal fluctuations used do not impact the viability of *D. melanogaster* and we failed to find a significant difference in the number of capsules (resistance of *D. melanogaster* to *L. boulandi*) between the three thermal regimes. However, we have shown that the success of *L. boulandi* parasitism (which represents a part of its virulence against *D. melanogaster*) increases with the thermal variability. Under the current global warming resulting from both increases in average temperature and of temperature variations (Easterling et al., 2000; Tebaldi et al., 2006; Zhou et al., 2009), we can expect a stronger negative impact of *L. boulandi* on *D. melanogaster* populations that may result in a demographic decline of *D. melanogaster* populations. To explore this question, it would thus be interesting to extend this study by investigating the impact of such small thermal fluctuations on other life history traits of *D. melanogaster* and also on the other host species of *L. boulandi*, *D. simulans*.

4.3. Underlying physiological mechanisms

Several experimental studies have shown that the use of fluctuating thermal regime relative to constant one alter the response of life history traits in insects, and especially the survival (Colinet et al., 2007; Kostal et al., 2007). One suggested explanation is that the injuries caused at low temperature are repaired during the periods at warmer temperatures (Colinet et al., 2007). One of the physiological processes that could explain the positive impact of fluctuating thermal regimes is the restoration of the ionic balance. Indeed in insects, exposure to cold temperatures results in the disturbance of ion homeostasis (Kostal et al., 2006; MacMillan et al., 2012). The warming periods experienced by insects under fluctuating temperature would allow reestablish the ionic balance and repair the damages caused by osmotic and ionic stress (MacMillan et al., 2012). Another hypothesis assumes that certain cold hardening processes that were interrupted at lower temperatures can be accomplished during recovery periods (Hanc and Nedved, 1999). Low temperature exposure may indeed hinder the gene transcription, mRNA splicing and protein translation and folding, leading to an increase in the number of aberrant proteins. Yet, several studies have demonstrated an up-regulation of Hsp proteins during the recovery period, that may prevent the dis-aggregation of proteins denatured by the cold and help their repair (Feder and Hoffmann, 1999; Wang et al., 2006; Colinet et al., 2007). Although we do not know the thermal physiology of *L. boulandi*, we can hypothesize that the observed difference in the SP between fluctuating thermal regime and constant temperature is related to the processes mentioned above.

5. Conclusion

In this study, we investigated the effects of three thermal regimes with the same mean temperature; one constant regime and two fluctuating regimes on the *Drosophila-L. boulandi* parasitoid interaction. We have shown that daily temperature fluctuations do not affect the viability of the host while they have a significant impact on the expression of the parasitoid phenotypic traits and in particular on its success of parasitism. Our results are consistent with predictions based on Jensen's inequality and constitute the first example of this kind on a high trophic level ectotherm. Finally, since parasitoids are key regulators of insect populations and are widely used as biological control agents against many agricultural pests, our results show the importance of taking into account fluctuating temperature in all laboratory tests to estimate the performance of these natural enemies.

Vitae

The authors belong to the team Genetics and Evolution of Host-Parasite Interactions (<http://lbb.e.univ-lyon1.fr/~Equipe-Genetique-Evolution-des.html>) of the Biometry and Evolutionary Laboratory (CNRS – University Lyon1). The authors are interested in the impacts of environmental change (invasive species, current climate change etc.) on species and communities, and particularly that on host-parasitoid systems.

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