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Temperature niche shift observed in a Lepidoptera population under allochronic divergence

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Abstract

A process of adaptive divergence for tolerance to high temperatures was identified using a rare model system, consisting of two sympatric populations of a Lepidoptera (*Thaumetopoea pityocampa*) with different life cycle timings, a 'mutant' population with summer larval development, Leiria SP, and the founder natural population, having winter larval development, Leiria WP. A third, allopatric population (Bordeaux WP) was also studied. First and second instar larvae were experimentally exposed to daily-cycles of heat treatment reaching maximum values of 36, 38, 40 and 42 °C; control groups placed at 25 °C. A lethal temperature effect was only significant at 42 °C, for Leiria SP, whereas all temperatures tested had a significant negative effect upon Leiria WP, thus indicating an upper threshold of survival c.a. 6 °C above that of the WP. Cox regression model, for pooled heat treatments, predicted mortality hazard to increase for Leiria WP (+108%) and Bordeaux WP (+78%) in contrast to Leiria SP; to increase by 24% for each additional °C; and to decrease by 53% from first to second instar larvae. High variability among individuals was observed, a population characteristic that may favour selection and consequent adaptation. Present findings provide an example of ecological differentiation, following a process of allochronic divergence. Results further contribute to a better understanding of the implications of climate change for ecological genetics.

Introduction

Speciation processes take a central role in ecology but still remain insufficiently studied (e.g. Nosil *et al.*, 2009). Sympatric speciation in particular, i.e. divergence without geographical isolation, is poorly understood although the topic has been attracting increased attention (e.g. Dieckmann & Doebeli, 1999; Simon *et al.*, 2000; Friesen *et al.*, 2007; Knudsen *et al.*, 2010). Furthermore, although some studies address sympatric speciation resulting from a diet switch or habitat specialization (e.g. Knudsen *et al.*, 2010), allochronic differentiation, a particular case in which asynchronous reproductive periods lead to speciation, while the evolved populations remain and coexist in the same area (Alexander & Bigelow, 1960), has scarcely ever been observed.

Allochronic speciation may be due to a temporal shift between populations reproducing in different years (e.g. Simon *et al.*, 2000; Cooley *et al.*, 2001; Ritchie, 2001), in different seasons (Friesen *et al.*, 2007; Santos *et al.*, 2007; Yamamoto & Sota, 2009) or even at different times of the day (Miyatake *et al.*, 2002). However, studies mostly describe the mechanisms of reproductive isolation through time, failing to clarify the resulting ecological and evolutionary consequences, at organism or population level. Yet, as a result of a life cycle phenological shift, a significant alteration of the environmental parameters driving selection pressures, such as temperature, natural enemies or food quality, among others, may occur. This

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can induce ecological differentiation of sister populations, by acting on traits that were not primarily involved in genetic differentiation.

The pine processionary moth, *Thaumetopoea pityocampa,* is a monovoltine conifer defoliator, widespread in the Mediterranean region. Adults emerge in the summer, mate and lay eggs within 2–4 days. The gregarious larvae hatch after ca. 1 month and development progresses until late winter, with the larvae sheltering inside self-spawn silk nests. Pupation takes place in the soil, where the pupae undergo an obligatory diapause. *T. pityocampa* exhibits a variable phenology across its range of distribution, with late adult emergence (August–September) and early pupation (January–February) occurring in the warmest regions, whereas early adult emergence (June–July) coupled with late pupation (March–April), is observed in populations colonizing the northernmost, or high-altitude areas (Démolin, 1969b). In 1997, a population of *T. pityocampa* having a shifted life cycle was discovered in Mata Nacional de Leiria (National Forest Park of Leiria) (M. R. Paiva, personal observation). In this population, hereafter called SP (summer population), the larvae develop in the summer, pupate in September and the adults reproduce in April–May. The SP was observed colonizing the same stands and host trees as individuals having the normal phenology of the winter population, hereafter called WP.

Previous research conducted on the genetics of both populations, showed that Leiria SP probably originated from Leiria WP in recent historical times, and that a possible mutation induced, in some individuals, a sudden phenological shift (Santos *et al.*, 2007, 2011). The two sympatric populations share the same major haplotype for the mitochondrial cytochrome oxidase 1, and one allele of the nuclear ITS1 marker which is endemic in Leiria region and separated from other *T. pityocampa* populations of the Iberian Peninsula. This fact excludes the hypothesis of Leiria SP being a cryptic species (Santos *et al.*, 2007) and suggests the occurrence of a fairly recent differentiation. Estimating the date of divergence between the two populations would yet require further molecular data. Gene flow is severely hampered between the two sympatric populations that are kept reproductively isolated by separate mating seasons (Santos *et al.*, 2011).

Following the phenological shift, the 'mutant' Leiria SP became suddenly exposed to new climatic conditions. In particular, larval development shifted from winter to summer, so that in Leiria, between June and September, SP larvae are subjected to daily temperatures on average 6.6 °C higher than those of the WP (Leiria Meteorological Station, Portugal).

Forecasts of population trends under different climate change scenarios (e.g. Crozier, 2004; Hickling *et al.*, 2006; Parmesan, 2006) evidence that temperature affects the ecology and biology of animal populations. Most studies focused on the description of observed shifts in geographic distributions (e.g. Denlinger & Yocum, 1998; Hickling *et al.*, 2006; Parmesan, 2006). Niche conservatism is generally implicitly hypothesized (Thuiller *et al.*, 2005) by taking into account the species current ecological requirements, in particular the realized niches thresholds. Nevertheless, niche divergence in space and time has also been predicted (e.g. Pearman *et al.*, 2008).

Different mechanisms allow insects to overcome unfavourable temperatures, namely physiological and biochemical adaptations, such as up-regulating heat-shock proteins (Denlinger & Yocum, 1998; Angilletta *et al.*, 2002); behavioural changes, such as micro-habitat selection (Breuer *et al.*, 1989), or aggregation (Jones & Oldroyd, 2006); and phenological alterations, mainly through the regulation of diapause induction or termination (Pullin, 1996; Bale & Hayward, 2010). Such mechanisms are, at least in part, under genetic regulation and could therefore evolve and enable adaptation to new environmental conditions. Within this context, functional genetic diversity is of particular importance, especially when it gives rise to phenotypic polymorphism.

A rare system, consisting of two sympatric *T. pityocampa* populations, SP and WP, was used to investigate the possible evolution of larval survival under extreme warm temperatures and to test the hypothesis that ecological requirements may evolve within a shorter than usually considered timeframe, such as tenths of generations. Experiments were complemented with data from another WP population, originating from France, where larval development starts in the summer. Larvae should thus be expected to tolerate higher temperatures than those of Leiria WP.

The specific objectives of this study were to establish if: (i) high-temperature survival would differ between the two *T. pityocampa* Leiria populations, SP and WP, thus indicating a process of ecological niche divergence; (ii) tolerance to high temperatures would differ between two 'normal' WP populations, sampled from distant regions at different latitudes; (iii) high-temperature tolerance might be considered a polymorphic trait, enabling selection to act.

Materials and methods

Larval origin

Thaumetopoea pityocampa larvae were obtained from eggs originating from three populations: (i) the unique Leiria SP (39°50′N; 8°57′W; < 50 m elevation); (ii) the sympatric Leiria WP and (iii) a natural population from Bordeaux, France (hereafter, Bordeaux WP), located at a higher latitude (44°44′N; 0°46′W; < 50 m elevation) than Leiria. The three populations were chosen because of their different phenologies, being hence exposed to different ecological conditions, particularly during the early larval stages. By contrast, they occur on the same hosts, namely maritime pine *Pinus pinaster* Ait., at a similar altitudinal range (30–50 m) and identical distance from the Atlantic Ocean. In the field, larval development starts in June for Leiria SP, in August for Bordeaux WP and in October for Leiria WP. In consequence, the average maximum temperatures (MT) (±SE) experienced by the young larval stages (first to third instars) are 25.2 ± 0.3, 21.7 ± 0.7 and 23.7 ± 0.7 °C, respectively for Leiria SP, Leiria WP and Bordeaux WP (Leiria Meteorological Station, 2002–2009; Bordeaux Merignac Meteorological Station, 2002–2010).

To minimize larval mortality because of environmental factors other than temperature, such as diseases and parasitism, as well as to standardize acclimatization conditions, egg batches were kept in the laboratories of ISA (Lisbon) at room temperature (25 ± 2 °C), until the larvae hatched. In all experiments, first and second instar larvae only were used (coded L1 and L2), because these stages are considered to be the most susceptible to extreme warm temperatures. L1 designates the larval stage running from hatching to the first moult, and L2 refers to the following one, ending with the second moult. L2 larvae were obtained from L1 larvae kept at room temperature and fed on maritime pine until the first moult.

Rearing temperature conditions

The experimental design was based on the temperature record for the summer months in Leiria region, between 2002 and 2009, during which 13 periods of hot summer days with daily MT exceeding 35.5 °C were observed, one of which lasted for three consecutive days (Fig. 1). Each treatment consisted of three complete 24 h temperature cycles, 16L : 8D, mimicking three consecutive extremely hot days, to test the effects of extreme MT on larval survival.

During the 8 h of scotophase, temperature was kept 10 °C below the tested MT; it was then gradually increased, from the beginning of the photophase, to reach the desired MT within 6 h. Temperature was kept constant for the following 4 h, corresponding to the expected length of the warmest daily period in the field, and progressively decreased during the remaining 6 h (Fig. S1 in Supporting Information). Preliminary experiments were conducted by placing Leiria SP larvae for 4 h at a constant temperature of 38 °C, followed by 3 h of larval survey. Surprisingly, only 11 of 98 larvae died under these conditions, whereas 5% died in the control group, so that the MTs used for the present work were then set at 36, 38, 40 or 42 °C.

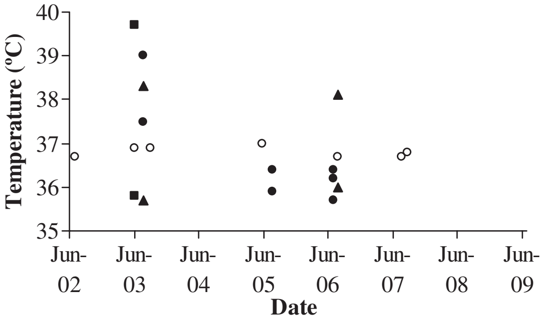

Fig. 1 Daily maximum temperatures above 35.5 °C recorded in Leiria Meteorological Station, Portugal, from June to September, 2002 to 2009. Within the same year, two or more consecutive days of extreme temperatures are indicated with the same filled pattern; open circles represent single hot days.

Prior to the start of the first cycle, the larvae were acclimatized, for 1 h to the initial temperature, i.e. 10 °C below the respective MT. All cycles started at the beginning of the scotophase. Experiments were conducted in a climatic chamber Fitoclima, model 700 EDTU (ARALAB, Rio de Mouro, Portugal), with relative humidity fixed at 60%.

Experimental design

Larvae were kept inside acrylic boxes (18 × 12 × 12 cm), and fed daily with *P. pinaster* needles attached to a freshly cut branch, inserted into wet floral foam (Oasis®; Smithers-Oasis, Maharashtra, India). Pine branches were previously sterilized by washing with a 0.53% bleach solution, rinsed and gently dried.

Each trial consisted of test boxes, exposed for three 24 h cycles to one of the four MTs, as described earlier, and of the matching control boxes kept at room temperature in the laboratory (25 ± 2 °C), for the same period. Room temperature was within the range of optimal temperature for larval development (Démolin, 1969a). Each replicate consisted of a test box and its corresponding control, comprising a sample of 20 larvae, collected at random from a mix of larvae hatched from five to seven egg batches (150–250 larvae usually hatch from each egg mass). In this way, treatment and control boxes were clearly associated and consisted of 'sister' groups of larvae of the same age and genetically closely related (although not from a single brood).

Four independent trials were conducted for each population and instar, according to the four MTs. On each trial, the number of test boxes equalled the number of controls, which varied between trials, depending on the availability of larvae. Numbers for MT = 36, 38, 40 and 42 °C were, respectively, (10, 10, 14, 14) L1 and (7, 17, 12, 7) L2 for Leiria SP; (7, 22, 7, 7) L1 and (12, 7, 7, 8) L2 for Leiria WP; (7, 7, 7, 7) L1 and (7, 7, 7, 5) L2 for Bordeaux WP. Trials for Leiria SP and Leiria WP were repeated in 2008 and 2009 and then pooled. All other trials were conducted in 2009 only.

Dead larvae were counted daily and removed from the climatic chamber, fresh needles were added and the Oasis® foams rehydrated. After three 24-h cycles, the boxes were transferred to the laboratory to the same temperatures as during the pretreatment period, and the larvae fed for an additional post-treatment period of 1 week. During this period and until the end of the trial, fresh pine needles were added and the Oasis® foam rehydrated every 2nd day. Post-treatment survival was recorded every 2nd or 3rd day.

Data analysis

Kaplan–Meyer mean survival (±SE) were estimated for each experiment (Gehan, 1975), and the respective survival curves plotted. Survival analyses were performed using Log-Rank (Mantel–Cox) test statistics in which all larvae that were still alive at the end of the experiments were considered as censored data (in survival analyses, censored data are observations for which death is not recorded). For each trial, statistical tests were applied to compare survival curves of test boxes to their corresponding controls. Tests were also performed to compare the survival of instars at each temperature for each population (i.e. L1 vs. L2 at a given MT). A Cox regression analysis, which provides robust inference for the regression coefficients of a hazard function dependent on explanatory variables (Lin & Wei, 1989), was further used to model the effects of temperature, instar and population, pooling heat treatments (36–42 °C). The hazard function $h_i(t)$, that is the potential for a death to occur at a particular time, was modelled assuming the equation:

$$h_i(t) = h_0(t) \exp\left(b_0 + b_1 \cdot \text{temperature} + b_2 + b_3\right)$$

where: $h_0(t)$ is the baseline hazard at time t; b_j is the value of the jth regression coefficient, as follows: 1 – temperature (°C); 2 – instar (L1 = 0; L2 = 1); 3 – population (population 1: Leiria WP = 1, population 2: Bordeaux WP = 1) reference category Leiria SP.

For each population and MT, the differences in the proportion of larvae surviving between treatment boxes and respective controls were further compared between populations at day 3, corresponding to the end of the heat treatment period, and at day 11, the end of the post-treatment period. To reach this aim, we performed two-way ANOVAS using two factors, namely ‘population’ with three modalities (Leiria SP, Leiria WP and Bordeaux WP) and ‘temperature’ with four modalities corresponding to the four MTs. A *post hoc* LSD test was used to compare population pairs. Different ANOVAS were used for each instar at day 3 and day 11. Prior to the ANOVA analysis, the homogeneity of variances was tested by the Levene’s test.

Results

Comparison of heat treatment effects within each population and larval instar

The effect of exposure to high temperatures was assessed by first comparing the survival curves for each experiment (for any given combination of population–instar–MT) with the respective controls. Results are summarized in Table 1.

Concerning Leiria SP, a significant negative temperature effect was observed for the highest MT only (42 °C). No significant effect was detected either at 38 or at 40 °C, for any of the instars. Unexpectedly, survival was significantly higher at MT = 36 °C for L1 larvae, in comparison with the corresponding control (Table 1, Fig. 2a).

Concerning Leiria WP, L1 were negatively affected by all tested MTs, including 36 °C, whereas L2 had a significantly lower survival at MT = 40 °C and MT = 42 °C. No significant effect of MT = 36 °C or MT = 38 °C on L2 survival occurred. Finally, for Bordeaux WP, survival was significantly lower only for L1 at MT = 38 °C and MT = 42 °C, and for L2 at MT = 38, 40 and 42 °C.

High variability at individual level was encountered in all groups (Fig. 2a,b). In all experiments, a gradual decrease in survival was observed over the treatment and post-treatment periods, rather than a sudden increase in mortality rate. For MT = 42 °C, between 5% and 75% of the individuals survived until the end of the post-treatment period, depending on the population and instar (Fig. 2b). However, L1 larvae from Leiria WP, exposed at

Table 1 Results of the Log Rank (Mantel–Cox) tests comparing the survival curves of larvae at each tested MT to the corresponding controls, Wald qui-square (d.f. = 1) and *P* values, for each population and instar, from the beginning of heat treatment until the end of the post-treatment period, 2008–2009.

MT	36 °C		38 °C		40 °C		42 °C	
Instar, population	Wald	*P*	Wald	*P*	Wald	*P*	Wald	*P*
First instar								
Leiria SP	6.59	***0.010***	0.55	0.459	1.41	0.236	114.70	**0.000**
Leiria WP	19.57	**0.000**	68.65	**0.000**	21.01	**0.000**	19.35	**0.000**
Bordeaux WP	0.00	0.964	3.92	**0.048**	2.96	0.085	60.40	**0.000**
Second instar								
Leiria SP	1.72	0.190	2.44	0.118	1.17	0.280	13.95	**0.000**
Leiria WP	3.44	0.063	1.68	0.194	4.21	**0.040**	67.04	**0.000**
Bordeaux WP	1.77	0.183	6.57	**0.010**	7.16	**0.007**	10.69	**0.001**

Bold regular: significant negative effect of the heat treatment; bold italic: significant positive effect of the heat treatment.

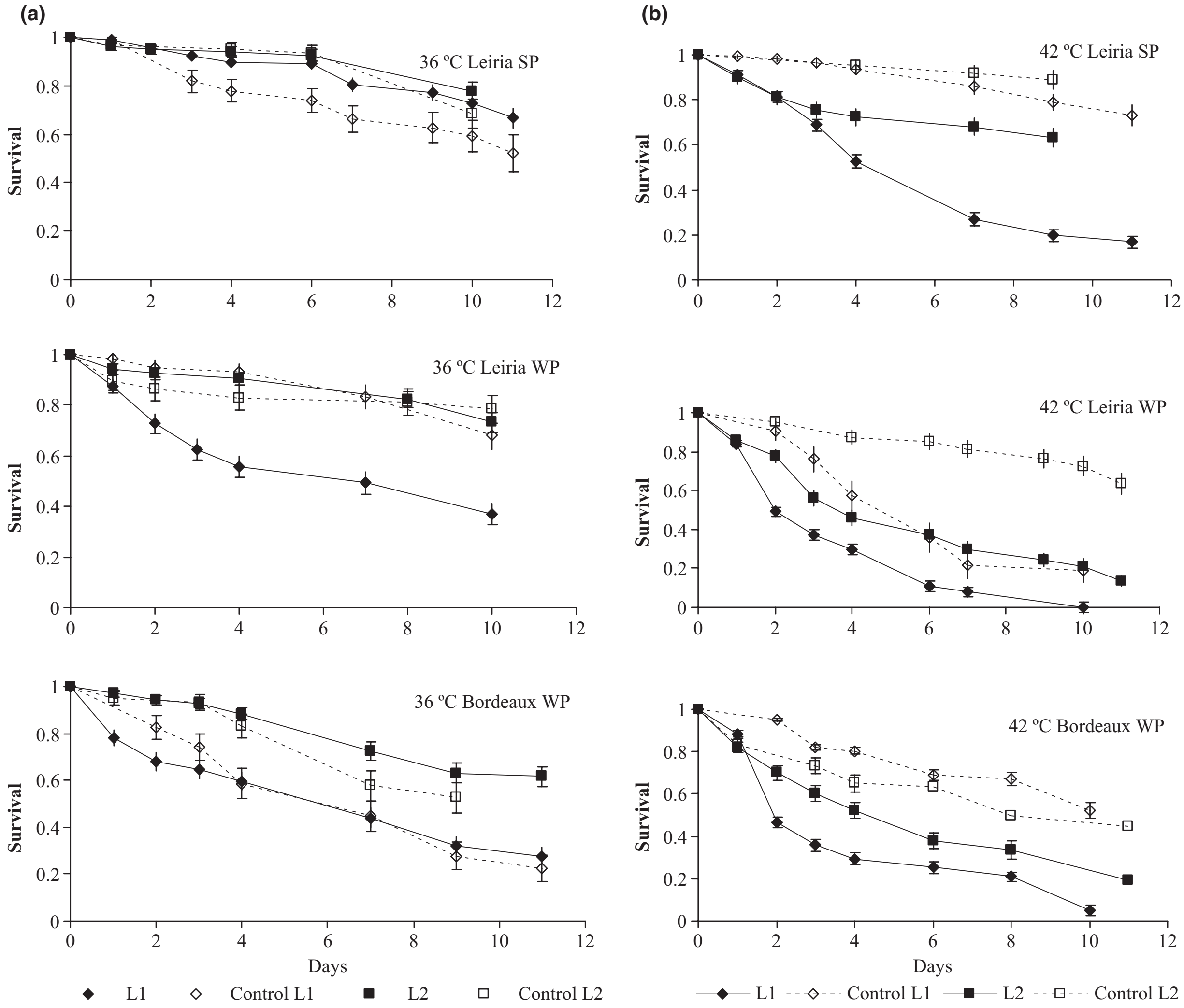

Fig. 2 Kaplan–Meyer survival estimates (±SE) for *Thaumetopoea pityocampa* L1 and L2 instar larvae, originating from Leiria SP (LSP), Leiria WP (LWP) and Bordeaux WP (BWP), subjected to temperature treatments: (a) MT = 36 °C and corresponding controls. (b) MT = 42 °C and corresponding controls.

MT = 42 °C, proved to be an exception because 100% mortality was observed after day 10 (Fig. 2b).

Comparison of heat treatment effects between instars and among populations

Results of the statistical analysis performed showed that L1 were in most cases more susceptible to high temperatures than L2, having a significantly lower survival at a given MT. Yet, for Leiria SP, both instars survived similarly at MT = 36°C and MT = 38 °C (Table 2).

Survival decrease (i.e. the difference in survival rate between treatment and control) was significantly higher in Leiria WP than in Leiria SP, for L1 at day 3 ($F_{2,112} = 13.88$; $P < 0.001$) and for L1 and L2 at day 11 ($F_{2,112} = 4.31$, $P = 0.016$ for L1; $F_{2,93} = 3.36$, $P = 0.039$ for L2) (Fig. 3). No significant difference was found for L2 at day 3 when comparing Leiria SP with Leiria WP ($F_{2,93} = 1.29$; $P = 0.281$). Concerning Bordeaux WP, the decrease in survival was ranked between Leiria WP and Leiria SP (Fig. 3). Because the interaction term temperature*population was in general not significant (L1 at day 3 – $F_{6,112} = 2.10$; $P = 0.059$; L2 at day 3 – $F_{6,93} = 1.82$; $P = 0.103$; L2 at day 11 – $F_{6,93} = 1.36$; $P = 0.240$), except for L1 at day 11 ($F_{6,112} = 9.44$; $P < 0.001$), the differences between populations for each MT were tested for this particular case only. For all MTs, except MT = 42 °C, decrease in survival was higher for Leiria WP than Leiria SP, Bordeaux WP always ranked in the middle, as shown in Fig. 3 for pooled values. For Leiria WP MT = 42 °C, an unexplained high mortality

Table 2 Results of the Log-Rank tests comparing survival rates of L1 and L2 *Thaumetopoea pityocampa* larvae for each maximum temperature (MT) and population tested, Wald qui-square (d.f. = 1) and *P* values.

MT (°C)	Population	Wald	*P*
36	Leiria SP	2.92	0.087
	Leiria WP	41.77	**0.000**
	Bordeaux WP	39.30	**0.000**
38	Leiria SP	0.26	0.609
	Leiria WP	72.81	**0.000**
	Bordeaux WP	17.09	**0.000**
40	Leiria SP	10.77	**0.001**
	Leiria WP	15.21	**0.000**
	Bordeaux WP	14.75	**0.000**
42	Leiria SP	53.63	**0.000**
	Leiria WP	32.61	**0.000**
	Bordeaux WP	23.10	**0.000**

Bold regular: Survival rate of L1 is significantly lower than survival rate of L2.

Table 3 Cox regression results for the pooled heat treatments data (36–42 °C): Hazard function regression coefficients (*B*) and standard error (in brackets), and the respective mortality hazard increase.

Variable	*B*(SE)	Wald d.f. = 1	*P*	Exp(*B*)	Mortality hazard (%)
Temperature (°C)	0.22 (0.01)	382.3	0.000	1.243	+24
Instar (L2 vs. L1)	−0.42 (0.04)	92.7	0.000	0.655	−53
Leiria WP vs. Leiria SP	0.73 (0.05)	201.8	0.000	2.079	+108
Bordeaux WP vs. Leiria SP	0.58 (0.06)	107.3	0.000	1.779	+78

was also observed in the control group, so that results cannot be coherently interpreted.

The Cox regression model to predict mortality hazard in function of the covariables temperature (ranging from 36 to 42 °C), instar and population revealed that all coefficients were significant (Table 3). According to the model, the mortality hazard increased by 24% for each additional °C, whereas from the first to the second larval instar it was reduced by 53%. The highest total increase in mortality is predicted for Leiria WP in comparison with Leiria SP (108%), whereas for Bordeaux WP this increase was 78% (Table 3).

Discussion

The present study characterized larval survival at high temperatures for a 'mutant' *T. pityocampa* population, recently founded by individuals originating from a typical winter population from Leiria (WP) (Santos *et al.*, 2007). Because of an abrupt phenological change (Santos *et al.*, 2007, 2011), the early larval instars of this summer population (SP) are now subjected to considerably higher temperatures than before. This unique situation allows for the study of rapid ecological divergence between two populations under allochronic differentiation. It is worth recalling that divergent natural selection, acting either by selection on a single trait or on a larger number of traits, can be an important factor further promoting ecological speciation (Nosil *et al.*, 2009). However, the occurrence of ecological divergence following genetic differentiation has hardly ever been documented (Savolainen *et al.*, 2006).

Manipulative laboratory experiments with short life cycle species, such as fruit flies, bacteria and fungi, reared for several generations under contrasting environments revealed that constraints can drive the rapid evolution of ecological traits (Bennett & Lenski, 1999; Garland & Kelly, 2006). Further, a genetic basis for heat tolerance was inferred from laboratory selection trials with *Drosophila* species (e.g. Huey *et al.*, 1991; Cavicchi *et al.*, 1995; Hoffmann *et al.*, 1997). However, regarding populations with longer life cycles, the knowledge gap is substantial.

The evidence now presented comes from a natural situation, for a species with a longer life cycle. By comparatively studying survival at high temperatures

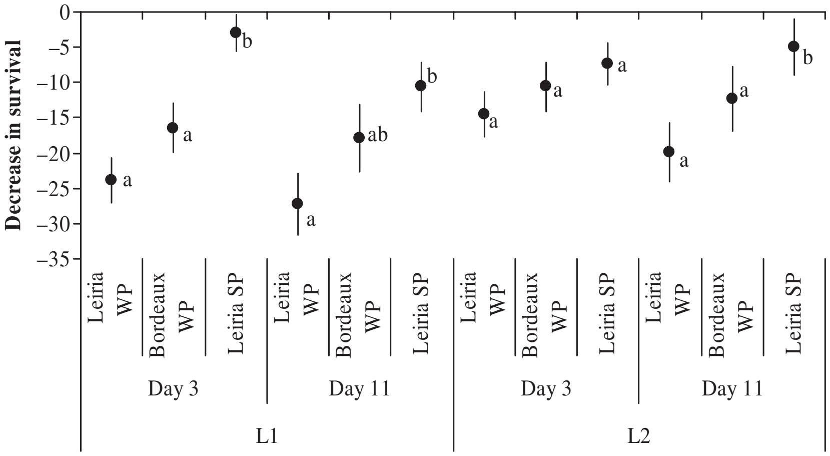

Fig. 3 Comparison of mean decreased in survival (±SE), i.e. difference in observed survival between treatment and control for all tested MTs, at day three (end of temperature treatment) and day 11 (end of the post-treatment period) between Leiria SP (LSP), Leiria WP *Thaumetopoea pityocampa* L1 and L2 larvae.

in two *T. pityocampa* winter populations, from regions located at different latitudes, Leiria and Bordeaux, which evolved under different selection pressures and have slightly different phenologies, it was concluded that tolerance to high temperatures is a rapidly evolving selected trait, showing high variability at individual level.

Population divergence for temperature tolerance thresholds: adaptation and possible rapid evolution

Thaumetopoea pityocampa larval survival differed among three populations tested, regarding tolerance to high temperatures. Tolerance was highest for Leiria SP and lowest for Leiria WP. Results are in accordance with the range of temperatures recorded locally, at the time of larval development, for each population and region: Leira SP, July–September, average MT (a.m.t.) 25.2 °C; Bordeaux WP, August–October, a.m.t. 23.7 °C; Leiria WP, September–November, a.m.t. 21.8 °C. L1 larvae from Leiria SP are further subjected to MTs exceeding 35.0 °C, a temperature to which no other population from *T. pityocampa*, or from its closely related *T. wilkinsoni* is normally exposed. In Mediterranean areas with very hot summers, such as Northern Africa, or the Middle East, oviposition occurs in October and consequently larval development takes place under cooler conditions. At these lower latitudes, pupae spend the summer in the soil, where temperatures are lower, this stage also being less susceptible than all the others to extreme temperatures (Pullin, 1996).

A survival rate of over 60% was observed at 36 °C for L1 larvae of the three populations, in contrast with data for south-eastern France, where L1 larvae experienced 100% mortality when daily temperatures reached 32 °C (Démolin, 1969a). Here, differences in temperature tolerance could be because of local adaptation, as larval development in south-eastern France starts under moderate temperatures, in the autumn. Survival at 36 °C was even higher than at control temperature, an unexpected result that could be because of a sterilization effect by the high temperature, upon pathogens that normally infect the larvae (Blanford & Thomas, 1999).

In this study, *T. pityocampa* individuals were sampled from and reared on *P. pinaster*, the most frequent host in Portugal and south-western France. The ecological differences observed are thus not due to a host effect and can be interpreted as local adaptation to climate by the early larval stages. Moreover, results show that variability exists at individual level, as mortality occurred throughout the experiments with some individuals dying very early after exposure to high temperatures, whereas others survived at least for 1 week after the end of the experiment.

Present results suggest that thermal tolerance was quickly acquired by a cohort of *T. pityocampa* SP founding individuals, from which enough survived to secure population viability. The mechanisms underlying these observations still need to be investigated. However, within population variability in tolerance to high temperatures is a trait that could rapidly evolve under strong evolutionary constraints (e.g. Thuiller *et al.*, 2005). It can be hypothesized that physiological adaptations should be involved, such as divergences in the genetic mechanisms up regulation heat-shock and stress-related genes, as observed in other insects (Mahadav *et al.*, 2009). To determine the occurrence of possible pleiotropy between phenology and thermal tolerance would necessitate further quantitative genetics, or genomic studies.

Phylogeographic studies have shown that *T. pityocampa* and *T. wilkinsoni* are genetically highly structured in space at different spatial scales (Kerdelhué *et al.*, 2006, 2009; Simonato *et al.*, 2007; Rousselet *et al.*, 2010). Results demonstrated the existence of local adaptation to temperature, suggesting that it probably occurs within the whole range of the species complex, and that this trait is variable for different populations and different regions where local climatic conditions differ. Phenotypic characteristics observed in one population should thus not be extrapolated to other populations. The same may apply to other traits, such as cold tolerance.

Implications for modelling population expansion

Summer temperatures in Leiria region are, on average, 4 °C lower than in neighbouring areas located 30 km inland, a fact which may have favoured the maintenance and development of the 'mutant' Leiria SP, and could explain that it remains confined to a quite narrow geographical range. The highest MT observed in Leiria, between 2002 and 2009 was 39.7 °C, in 1 day only (Fig. 1). Nevertheless, larval survival of Leiria SP was not affected by temperatures up to 40 °C, and about 20% and 70%, respectively of the L1 and L2 individuals, still survived for three consecutive days at a MT of 42 °C. This suggests that adaptation to hotter summers can be expected and that temperature constraints might not preclude SP expansion beyond coastal areas, into more continental or Mediterranean regions.

Models forecasting the dynamics of insect species under changing scenarios generally use parameters extrapolated from one or a few locations only. Models usually underestimate, or even ignore geographic variations of adaptive traits and thus of the realized niche breadth among populations (Sutherst & Maywald, 1985; Baker *et al.*, 2000), which can be relatively large, especially near range boundaries (Oliver *et al.*, 2009). From an applied perspective, present results draw attention to the fragility of forecasts assuming spatial and/or temporal niche conservatism (e.g. Thuiller *et al.*, 2005), without considering evolutionary processes.

In conclusion, this study evidences that rapid adaptation to higher temperatures is most likely to occur in insect species. In a field mutant population, an increase in 6 °C of the upper temperature threshold was observed to have evolved in a relatively short period of time. Results can find application within the context of climate change scenarios, suggesting that some populations may remain *in situ*, even when ecological parameters attain values surpassing the species thresholds of tolerance.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Climatic chamber 24 hour-temperature cycles. For each maximum temperature MT, trials correspond to three consecutive 24-h cycles.

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