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Evolution of metabolic rate in a parasitic wasp: The role of limitation in intrinsic resources

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ABSTRACT

Metabolic rate, a physiological trait closely related to fitness traits, is expected to evolve in response to two main environmental variables: (1) climate, low metabolic rates being found in dry and hot regions when comparing populations originating from different climates in a common garden experiment and (2) resource limitations, low metabolic rates being selected when resources are limited. The main goal of this study was to investigate if differences in intrinsic resource limitations may have disrupted the expected evolution of metabolic rate in response to climate in a parasitic wasp.

We compared CO_2 production of females from 4 populations of a *Drosophila parasitoid, Leptopilina boulardi*, as an estimate of their metabolic rate. Two populations from a hot and dry area able to synthesise lipids *de novo* at adult stage were compared with two populations originating from a mild and humid climate where no lipid accumulation during adult life was observed. These last females are thus more limited in lipids than the first ones.

We observed that a high metabolic rate has been selected in hot and dry environments, contrarily to the results of a great majority of studies. We suggest that lipogenesis occurring there may have allowed the selection of a higher metabolic rate, as females are less limited in energetic resources than females from the mild environment. A high metabolic rate may have been selected there as it partly compensates for the long distances that females have to cross to find laying opportunities in distant orchards. We suggest that intrinsic resources should be integrated when investigating geographical variations in metabolism as this factor may disrupt evolution in response to climate.

1. Introduction

Organisms consume energy from their environment, convert it within their bodies, allocate it to fitness-related traits such as fecundity, longevity or growth, and excrete altered forms back into the environment (Brown et al., 2004). All of these processes define metabolism. Metabolic rate (i.e. the rate of energy uptake, transformation and allocation), generally measured as O_2 consumption or CO_2 production, is thus closely related to the fitness of organisms. For example, an increase in metabolic rate is associated with an increase in growth rate (e.g. Metcalfe, 1998; Nylin and Gotthard, 1998) and a concurrent decrease in development time (Nylin and Gotthard, 1998), longevity (Huey and Stevenson, 1979; Artacho and Nespolo, 2009) or fecundity (Crnokrak and Roff, 2002) as energy used for metabolism is not available for these traits. Thus, understanding the evolution of metabolism is a central element in the comprehension of evolution of life histories.

Metabolism is a biological process that follows physical and chemical laws governing the transformations of energy and materials. Consequently, temperature is a major environmental factor affecting metabolic rate, particularly in ectotherms. An increase in metabolic rate when organisms are placed at higher temperatures, within a certain range (0–40 °C generally), has been universally predicted and observed (Clarke and Fraser, 2004; Gillooly et al., 2001; Nespolo et al., 2007). However, how metabolic rate evolves in response to climate is still highly debated, even if there is clear evidence that local adaptations to climate exist. Two main hypotheses on evolution of metabolic rate in response to climate have been described, leading to converging conclusions.

The most described is the Metabolic Cold Adaptation (MCA) hypothesis, also called Metabolic Compensation hypothesis (Conover and Schultz, 1995). The MCA hypothesis assumes that high metabolic rates have been selected in cold environments to compensate for the negative effect of low temperatures on

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metabolism. This would result in higher metabolic rates in populations or species from cold climates when compared with warm-adapted populations or species in a common garden, assuming that selection for metabolic rate has not affected thermal sensitivity. Several intraspecific and interspecific comparisons have argued in favour of MCA in fish (Alvarez et al., 2006; Cano and Nicieza, 2006), grasshoppers (Chappell, 1983; Massion, 1983), *Drosophila* species (Berrigan and Partridge, 1997), beetles (Strømme et al., 1986; Schultz et al., 1992) or more recently in parasitic wasps (Le Lann et al., 2011; Seyahooei et al., 2011), but others did not support it (Clarke and Johnston, 1999; Lardies et al., 2004; Lee and Baust, 1982; Nylund, 1991). Addo-Bediako et al. (2002) provided a strong evidence for the MCA hypothesis in a global-scale analysis on interspecific geographical variations of metabolic rate in insects.

The second hypothesis on evolution of metabolic rate in response to climate suggests that a low one should be selected in hot and dry environments such as deserts (Mueller and Diamond, 2001) as it confers resistance to high temperatures and desiccation by reducing exchanges with a stressful environment (Alvarez et al., 2006; Massion, 1983). Thus comparing populations from different climates reared in a common garden, low metabolic rate genotypes should be observed in hot and dry environments when compared with cooler and wet environments, according to the MCA hypothesis and to the hypothesis on resistance to desiccation.

A second important ecological factor in the evolution of metabolic rate is resource limitations. Indeed, a higher metabolic rate involves a higher amount of energy to maintain the body and thus fewer resources for fitness traits such as fecundity. Consequently, a lower metabolic rate is expected when resources are limited, to reduce the rate of resource consumption (Hoffmann and Parsons, 1997; Mueller and Diamond, 2001). In parasitic wasps, lipids represent the main energy resource allocated to survival, reproduction and dispersal (Ellers and van Alphen, 1997; Ellers et al., 1998; Rivero and Casas, 1999). These organisms were thought to be unable to synthesise lipids during adult life (for a review, see Visser and Ellers, 2008) but Visser et al. (2010) recently described lipogenesis in some species. Moiroux et al. (2010) found intraspecific variations in lipogenesis ability in adults, or at least in lipid accumulation, between populations of a *Drosophila parasitoid*, *Leptopilina boulardi* (Hymenoptera: Eucoilidae), originating from different climates. Females from an Iranian hot and dry area were able to synthesise lipids during adult life whereas a strong decrease in lipid quantity was observed in populations from the mild and humid Iranian coast of the Caspian Sea, suggesting that no lipogenesis occurred there. Thus, the differences in the limitation in intrinsic resources between populations from different climates may represent a difference in physiological constraints that is likely to have affected the evolution of metabolic rate. Indeed, the cost of maintaining a high metabolic rate on fitness-related traits should be stronger when resources are limited.

The main goal of this study was precisely to investigate if metabolic rate has mainly evolved in response to climate or was constrained by differences in intrinsic resource limitations. We thus compared the CO_2 production of females originating from a hot and dry climate and able to synthesise lipids with females from a mild and humid climate where no lipid accumulation during adult life was observed. Considering the above-mentioned literature, two patterns can be expected (1) if metabolic rate has been mainly selected in response to climate (MCA hypothesis and hypothesis on resistance to desiccation), we should observe a lower metabolic rate in populations from the hot and dry area; (2) if the evolution of metabolic rate was differently constrained by differences in resource limitation, we may observe a higher metabolic rate in populations from the hot and dry area as adult lipogenesis occurs there.

2. Materials and methods

2.1. Rearing

Four genetically distinct populations (Seyahooei, 2010) of *L. boulardi* (Barbotin, Carton & Keiner-Pillault, 1979), a solitary endoparasitoid that mainly attacks *Drosophila melanogaster* and *Drosophila simulans* larvae, were collected in July 2006 in Iran–two in a mild and humid region and two in a hot and dry area–using twelve banana bait traps per population. Each open trap (i.e. a plastic container with a 3 cm diameter hole covered with mesh with 2 mm openings) was colonised by five to twenty females and several hundreds of offspring were produced in each of them. From the offspring thirty females per population were taken to set up lab cultures. Two populations–Chalus and Seyakhal (called Mild humid 1 and 2 in next sections) – originated from a humid mild environment, in the coastal plain of the Caspian Sea. Dorcheh and Zamankhan strains (called Hot dry 1 and 2 in next sections) originated from a very dry, hot area close to Esfahan, although the location of our traps was in the valley of a snow-fed river that keeps water throughout most summers and allows fruit productions. Climatic data is presented in Table 1.

D. melanogaster used as hosts in the cultures originating from strains collected in the Netherlands in 1960. Parasitoids and fruit flies were reared at 25 ± 1 °C – optimal rearing temperature for *L. boulardi* considering the numbers of individuals that successfully emerged as adults – , 50 ± 10% RH and 16L:8D photoperiod.

For the experiment, *L. boulardi* females oviposited in separate jars for 12 h at 25 °C in 150 s instar *D. melanogaster* larvae laid in a 2 h period in a baker's yeast suspension. The parasitised larvae were then separated into four groups which were placed at 20, 22.5, 25 and 27.5 °C. The metabolic rate was measured at these four temperatures as different reaction norms may have been selected in different climates. We also measured the amount of lipids to check for the lipid accumulation during adult life in our populations with a more precise analysis method (see below) than used in a previous study (Moiroux et al., 2010). When emerging, wasps were separated into two groups. The first one was used for measuring metabolic rate and the second one for measuring lipids.

No individuals from Dorcheh emerged at 20 °C so no data is available at this temperature for this population. It is likely that diapause occurs at this temperature as observed in other populations of *L. boulardi* (Carton and Claret, 1982).

2.1.1. Metabolic rate

Routine metabolic trait – that corresponds to basal metabolic rate plus some contribution from activity – was measured at the same temperature at which parasitoids were reared (i.e. 20, 22.5, 25 or 27.5 °C). We used flow-through respirometry to measure metabolic rate of emerging female wasps. Fifteen to twenty samples per temperature were tested for each population. To detect a strong signal, twenty individuals were pooled per sample. After having their fresh mass measured with a microbalance Sartorius (±0.001 mg), fourteen samples of twenty 1-day old individuals were placed in a climate room at rearing temperature in separate small cylindrical chambers and their CO_2 production was measured with an infra-red CO_2 analyser (Li-COR LI6251). A fifteenth chamber was kept empty as a control. A Sierra mass flow controller maintained constant flow rates of dry, CO_2-free air. Air was drawn off from the environment and CO_2 and water were scrubbed with a Drierite–Ascarite column. Four records of 5 min for each sample were performed with a gap of 90 min between two recordings, and were automatically transformed by a programme recorded in DATACAN software (Sable Systems, Las Vegas), to convert the

Table 1
Climatic data recorded for the four sampled areas, from 1977 to 2005 for Chalus, 1955 to 2005 for Seyakhal and Zamankhan, 1951 to 2005 for Dorcheh (*Source:* Islamic Republic of Iran Meteorological Organisation). We considered the average of each parameter measured every day from April to September, which is the period of activity of *L. boulardi*. Thermal amplitude was calculated as the mean of the differences between maximum and minimum air temperature per month.

Sampling areas	Temperature (°C)	Thermal amplitude (°C)	Relative humidity (%)	Number of rainy days	Precipitation amount (mm)
Chalus	26.3	7.6	80	43.3	248
Seyakhal	26.4	9.8	79	56.7	433
Dorcheh	31.6	15.6	29.7	14.2	30.9
Zamankhan	28.6	19.2	36	13.8	55

measure from ppm to μL-CO_2 per hour, taking into account the flow rate. The first recording allowed individuals to get used to their new environment and was not considered for analysis. The value measured in the control chamber was subtracted to the values measured in chambers with wasps. We considered the average of the last three recordings as a measure of metabolic rate for each sample. Recordings were performed at regular hour during photophase (10:00 am–04:00 pm) and scotophase (6:00 pm–12:00 pm) to cover different periods of the day. Routine metabolic rate is generally measured during scotophase, when insects are inactive, however we observed strong differences in locomotor activity among the four populations during this period, females from the desert area being more active than females from the mild area (Moiroux et al., 2010). During the photophase, we did not observe any significant difference in the locomotor activity among populations. Measures of the metabolic rate were thus not mainly affected by differences in locomotor activity during this period and we may mainly thus consider differences as the result of differences in the basal metabolic rate.

2.1.2. Lipid analysis

The amount of lipids was investigated at three ages to detect lipid accumulation during adult life. Adult parasitoids used for measures of lipid quantity were reared at the same temperature than their larval development temperature, 12L:12D in glass jars on an Agar-Nipagine substrate, fed with acacia honey diluted to 20% and distributed *ad libitum*. We measured the lipid content of 20 females per age and temperature for each population, using a colorimetric analysis protocol developed for parasitoids by Giron et al. (2002), adapted from van Handel (1985). Twenty 1-day, 5-days and 10-days old females were individually placed in Eppendorf tubes, killed in liquid nitrogen and conserved at −80 °C. After defrosting, the length of the left hind tibia was measured (±0.01 mm) with the numeric image analysis software Pegasus Pro V4 under a binocular (Olympus SZ-6045TR) linked to a video camera (JVC KY-F). Then both ovaries were removed, as we were interested in stored lipids, and wasps were individually placed in Eppendorf tubes in 50 μL of Ringer solution and crushed with a plastic pestle in 300 μL of methanol. Tubes were centrifuged for 15 min at 4 °C and 1400 rpm. One hundred and fifty micro litre of chloroform and 60 μL of 2% sodium sulphate solution were then added to samples and tubes were vortexed and stored at 4 °C for one night. The next day, tubes were centrifuged for 15 min at 1400 rpm. Three hundred micro litre of supernatant per sample were transferred to other tubes that were put on an aluminium heat block at 90 °C until total evaporation was complete. Forty micro litre of 98% sulphuric acid were then added, tubes were heated again at 90 °C for 2 min and cooled down on ice. Nine hundred and Sixty micro litre of Vanillin Reagent were added and tubes were vortexed and left for 15 min at ambient temperature. The absorbance was read at 525 nm with a spectrophotometer (VersaMAX). The calibration curve we used to transform absorbance into concentrations was made with standard sunflower oil.

2.2. Statistical analysis

Metabolic rate increases with body size (Gillooly et al., 2001) so we performed ANCOVA with the sampling site as the fixed factor and fresh mass of the 20 individuals pooled per sample as the covariate to test differences in metabolic rate. Metabolic rate during photophase and scotophase were tested in separate models.

We also used analysis of covariance ANCOVA to analyse the effect of age on the amount of lipids with tibia length as the covariate for each population and each temperature, to detect lipogenesis. We performed Scheffe tests as post hoc analysis for both lipid and metabolic rate.

All analyses were carried out using R software version 2.8.1 (R Development Core Team, 2008).

3. Results

3.1. Metabolic rate

The sampling site had a significant effect on metabolic rate. Populations from the desert area generally had a higher metabolic rate than those from the mild, humid climate at every temperature, as well as at different times in the photoperiod (Fig. 1). Hot dry 1 and 2 populations had a higher metabolic rate than Mild humid 1 and 2 populations with fresh mass as covariate at 22.5 °C, 25 °C and 27.5 °C during photophase and scotophase (Table 2). Hot dry 2 population had a higher metabolic rate than mild humid 1 and 2 populations at 20 °C with light but no difference occurred in darkness (Table 2).

In every population, we observed a significant effect of rearing temperature on metabolic rate and there were no differences between populations in the shape of metabolic rate reaction norms (Fig. 1). The elevation was indeed different but the slopes were similar. Metabolic rate with light increased with temperature between 20 °C, 22.5 °C and 25 °C and decreased between 25 °C and 27.5 °C in every population (Mild humid 1: $df = 3, 68$, $F = 5.105$, $p = 0.015$, Mild humid 2: $df = 3, 74$, $F = 3.65$, $p = 0.018$, Hot dry 1: $df = 2, 50$, $F = 2.43$, $p = 0.044$, Hot dry 2: $df = 3, 71$, $F = 3.45$, $p = 0.027$). The same pattern was observed in darkness (Mild humid 1: $df = 3, 68$, $F = 1.92$, $p = 0.051$, Mild humid 2: $df = 3, 74$, $F = 2.93$, $p = 0.035$, Hot dry 1: $df = 2, 50$, $F = 3.912$, $p = 0.028$, Hot dry 2: $df = 3, 71$, $F = 0.442$, $p = 0.008$) except that no difference occurred between 20 °C and 22.5 °C for the populations from the mild humid area.

3.2. Lipid analysis

There was no evidence that females from the mild, humid environment accumulated lipids but did in those from the desert area (Fig. 2). We indeed observed a significant decrease in lipid quantity between emergence, 5 days and 10 days in females from the mild humid region reared at 20 °C, 22.5 °C, 25 °C and 27.5 °C (Table 3). At 22.5 °C and 25 °C, we observed a significant increase in lipid quantities in females from the hot and dry environment between emergence and 5 days but no difference was observed between

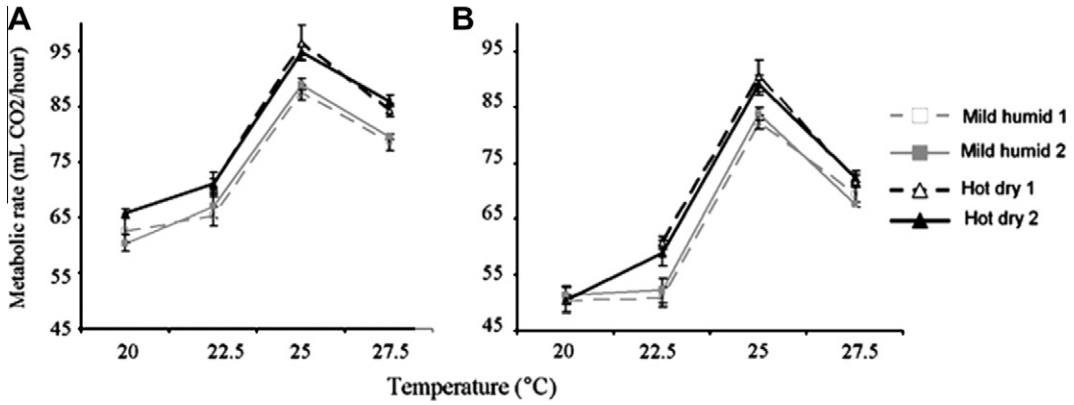

Fig. 1. Metabolic rate during photophase (A) and scotophase (B) measured at four temperatures for the 4 populations. Error bars: ±s.e. No individual from Dorcheh (Hot dry 1) emerged at 20 °C.

Table 2
Results of ANCOVA comparing metabolic rate of the 4 populations with the sampling site as the fixed factor and fresh mass as the covariate, during photophase or scotophase. Females from the desert area had a higher metabolic rate than females from the mild, humid area.

Temperature (°C)	Photophase	Scotophase
20	$df = 2, 52$, $F = 2.72$, $p = 0.045$	$df = 2, 52$, $F = 0.85$, $p = 0.43$
22.5	$df = 3, 72$, $F = 4.00$, $p = 0.012$	$df = 3, 72$, $F = 3.56$, $p = 0.02$
25	$df = 3, 72$, $F = 3.04$, $p = 0.01$	$df = 3, 72$, $F = 3.56$, $p = 0.02$
27.5	$df = 3, 62$, $F = 2.91$, $p = 0.036$	$df = 3, 62$, $F = 4.09$, $p = 0.019$

5 days and 10 days. No significant difference was observed at 27.5 °C in these females between emergence, 5 days and 10 days nor at 20 °C (Table 3).

Females from the mild and humid area emerged with more lipids than females from the hot dry environment at every temperature ($df = 7, 74$, $F = 4.89$, $p = 0.004$). The opposite pattern occurred at five ($df = 7, 72$, $F = 5.21$, $p = 0.01$) and 10 days ($df = 70$, $F = 6.80$, $p = 0.014$) of adult life.

4. Discussion

We observed that females living in the hottest and driest area had the highest metabolic rate, independently of rearing temperature or presence/absence of light, and thus of their locomotor activity level (Fig. 1). Our results are in contradiction with the great majority of studies on evolution of metabolic rate in response to climate where populations/species from temperate climates were found to have a higher metabolic rate than the ones from hot and/or dry climates (e.g. Addo-Bediako et al., 2002; Berrigan and Partridge, 1997). Authors suggested that high metabolic rates have been selected in cold environments to compensate for the negative effect of low temperatures on metabolism, or that low metabolic rates have been selected in hot and dry climates to limit effects of desiccation and stressful temperature (Massion, 1983). Contrary to these studies, our results suggest that geographical variations of routine metabolic rates have probably not been mainly selected in response to climate of origin in these parasitic wasps, but was strongly affected by the limitation in intrinsic resources.

Interestingly, we were able to show, as in a previous study (Moiroux et al., 2010), that lipogenesis occurs in populations from

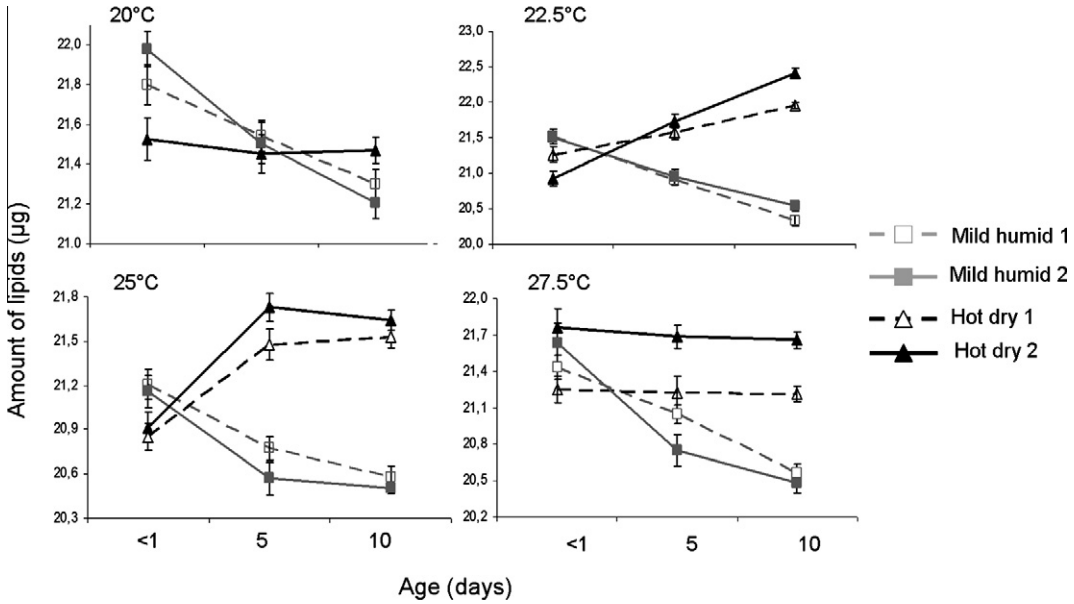

Fig. 2. Temporal variations in the amount of lipid for the 4 populations ($n = 20$ females/population). Error bars: ±s.e. No individual from Dorcheh (Hot dry 1) emerged at 20 °C.

Table 3
Results of ANCOVA comparing the effect of age on the amount of lipids with tibia length as the covariate for each population and each temperature, to detect lipogenesis. The amount of lipid significantly decreased with age in populations from the mild humid area while it increased or did not change in populations from the desert area.

Temperature (°C)	Populations			
	Mild humid 1	Mild humid 2	Hot dry 1	Hot dry 2
20	$df = 5, 56$, $F = 19.03$, $p < 0.001$	$df = 5, 56$, $F = 8.13$, $p < 0.001$	NA	$df = 5,54$, $F = 0.15$, $p = 0.923$
22.5	$df = 5, 56$, $F = 9.87$, $p < 0.001$	$df = 5, 56$, $F = 14.12$, $p = 0.002$	$df = 5, 54$, $F = 4.11$, $p = 0.023$	$df = 5, 56$, $F = 14.03$, $p < 0.001$
25	$df = 5, 54$, $F = 23.33$, $p < 0.001$	$df = 5, 56$, $F = 27.34$, $p < 0.001$	$df = 5, 56$, $F = 3.97$, $p = 0.037$	$df = 5, 56$, $F = 7.25$, $p < 0.01$
27.5	$df = 5, 50$, $F = 6.14$, $p = 0.017$	$df = 5, 52$, $F = 3.05$, $p = 0.047$	$df = 5, 52$, $F = 3.05$, $p = 0.094$	$df = 5, 54$, $F = 3.62$, $p = 0.01$

the hot and dry area and is unlikely to occur in populations from the mild humid area (Fig. 2). When feeding on carbohydrate sources such as nectar, females from the hot dry area produce lipids *de novo* at 22.5 °C and 25 °C whereas females from the mild climate probably do not, or at least consume them faster than they synthesise. At 20 °C and 27.5 °C, there was no clear evidence of lipogenesis in females from the hot dry area as no difference in lipid content with age was found. However, the lack of decrease with age in lipid content suggests that lipogenesis may also occur at these temperatures.

The consequence of these differences is that limitation in intrinsic resources is stronger in females from the mild climate even if, as observed in this study, they emerge with more lipids than females from the hot and dry region. Lipogenesis ability may have allowed the selection for a higher metabolic rate in the desert area, by affecting the energetic constrain of a trade-off between metabolic rate and fitness-related traits. This increase in metabolic rate occurs without shortening lifespan or decreasing egg load, as females from the dry area lived longer and produced more eggs than females from the mild area (Moiroux et al., 2010). It is likely that a higher metabolic rate may have not been selected in a hot and dry climate without lipogenesis ability, as the cost associated with a higher amount of energy to maintain organism would have strongly decrease the amount of energy available for fitness-related traits such as longevity and fecundity.

Lipogenesis ability may have allowed for the selection of a higher metabolic rate but it is probably not a factor that has selected for it. Having a higher metabolic rate has to be advantageous to be selected. In the desert area, parasitic wasps have to cross large distances because laying opportunities are concentrated in distant orchards distributed along a river. Moiroux et al. (2010) suggested that lipogenesis may have been selected in populations from the hot and dry area as a large amount of lipids is required to fly over large distances (Ellers et al., 1998). The present study advocates towards additional evolutionary advantages for lipid accumulation. The decrease in resource limitation may have changed physiological constraints there and allowed for the selection of a higher metabolic rate in this region, disrupting the expected evolution of metabolic rate in response to climate. Here, a high routine metabolic rate may be adaptive as it may allow wasps to rapidly produce ATP (Kolluru et al., 2004) when performing energetically costly flights (Brett and Groves, 1979; Cano and Nicieza, 2006) to find hosts. The increased locomotory activity would compensate for the long distances between patches of hosts in a dry area and could result in a higher number of encountered hosts, and therefore in a higher progeny.

Additionally, in the hot and dry environment, lipids accumulated during adult life may also limit desiccation (Hadley, 1994) and partially compensate for the additional water loss due to a higher metabolic rate (Clarke, 1993; Massion, 1983). Desiccation may also be limited in these populations by a higher activity rate during the night, as we observed in a previous study (Moiroux et al., 2010).

4.1. Reaction norm

Metabolic rate increased with temperature between 20 °C and 22.5 °C as found in the great majority of studies on temperature-dependence of metabolism. However we observed a decrease between 25 °C and 27.5 °C suggesting that this last temperature is physiologically stressful and may negatively affect the metabolism and activity of organisms. The strong decrease in the number of emergences between these two temperatures (from 80% at 25 °C in all populations to 50% at 27.5 °C in the dry area and 30% in the mild area) confirms that 27.5 °C is a stressful temperature. Moiroux et al. (2010) found that females from the hot dry area were more active at night. In this paper, authors suggested that this shift to nocturnal activity was adaptive as this behaviour would limit exposure to extreme diurnal temperatures. Results on metabolic rate at 27.5 °C tend to confirm that avoidance of high temperatures may be adaptive as these temperatures may be physiologically stressful.

Even if we observed a higher metabolic rate in populations from the hot region than in populations from the mild area, similar metabolic rate reaction norms were found in the four populations. Our results hence suggest that no selection on reaction norms of metabolic rate or on strength of phenotypic plasticity occurred. Metabolism obeys strict physical and chemical principles governing the transformations of energy and materials, among which the most relevant are the laws of mass and energy balance and thermodynamics (Brown et al., 2004). It is likely that no selection on the strength of phenotypic plasticity of metabolic rate apply as that would probably involve selecting for changes in these laws.

4.2. Conclusion

The evolution of metabolic rate, a central element in evolution of life history traits, has always been considered as the result of environmental factors, particularly temperature and environmental resource limitations or unpredictability. We suggest that differences in physiological constraints between populations may have affected this evolution and should be integrated when investigating geographical variations of metabolic rate.

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