

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

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1	Evolution of metabolic rate in a parasitic wasp: the role of		
2	limitation in intrinsic resources		
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14	Running headline: Lipogenesis affects the evolution of metabolic rate in parasitoids.		
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### 17 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 four populations of a Drosophila parasitoid, *Leptopilina boulardi*, as an estimate of their metabolic rate. Two populations from a hot and dry area able to synthesize 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.

30 We observed that a high metabolic rate has been selected in hot and dry environments, 31 contrarily to the results of a great majority of studies. We suggest that lipogenesis occurring 32 there may have allowed the selection of a higher metabolic rate, as females are less limited in 33 energetic resources than females from the mild environment. A high metabolic rate may have 34 been selected there as it partly compensates for the long distances that females have to cross 35 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 36 37 evolution in response to climate.

38

39 Key-words: lipogenesis, local adaptation, metabolic rate, parasitoids.

### 41 Introduction

42 Organisms consume energy from their environment, convert it within their bodies, allocate it 43 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. 44 45 Metabolic rate (i.e. the rate of energy uptake, transformation and allocation), generally 46 measured as  $O_2$  consumption or  $CO_2$  production, is thus closely related to the fitness of 47 organisms. For example, an increase in metabolic rate is associated with an increase in growth 48 rate (e.g. Metcalfe, 1998; Nylin and Gotthard, 1998) and a concurrent decrease in 49 development time (Nylin and Gotthard, 1998), longevity (Huey and Stevenson, 1979, Artacho 50 and Nespolo, 2009) or fecundity (Crnokrak and Roff, 2002) as energy used for metabolism is 51 not available for these traits. Thus, understanding the evolution of metabolism is a central 52 element in the comprehension of evolution of life histories.

53 Metabolism is a biological process that follows physical and chemical laws governing the 54 transformations of energy and materials. Consequently, temperature is a major environmental 55 factor affecting metabolic rate, particularly in ectotherms. An increase in metabolic rate when 56 organisms are placed at higher temperatures, within a certain range (0 to 40°C generally), has 57 been universally predicted and observed (Clarke and Fraser, 2004; Gillooly et al., 2001; 58 Nespolo et al., 2007). However, how metabolic rate evolves in response to climate is still 59 highly debated, even if there is clear evidence that local adaptations to climate exist. Two 60 main hypotheses on evolution of metabolic rate in response to climate have been described, 61 leading to converging conclusions.

62 The most described is the Metabolic Cold Adaptation (MCA) hypothesis, also called Metabolic Compensation hypothesis (Conover and Schultz, 1995). The MCA hypothesis 63 64 assumes that high metabolic rates have been selected in cold environments to compensate for the negative effect of low temperatures on metabolism. This would result in higher metabolic 65 rates in populations or species from cold climates when compared with warm-adapted 66 67 populations or species in a common garden, assuming that selection for metabolic rate has not 68 affected thermal sensitivity. Several intraspecific and interspecific comparisons have argued in favour of MCA in fish (Álvarez et al., 2006; Cano and Nicieza, 2006), grasshoppers 69 70 (Chappell, 1983; Massion, 1983), Drosophila species (Berrigan and Partridge, 1997), beetles 71 (Strømme et al., 1986; Schultz et al., 1992) or more recently in parasitic wasps (Le Lann et 72 al., 2011; Seyahooei et al. 2011), but others did not support it (Clarke and Johnston, 1999; 73 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.

76 The second hypothesis on evolution of metabolic rate in response to climate suggests that a 77 low one should be selected in hot and dry environments such as deserts (Mueller and 78 Diamond, 2001) as it confers resistance to high temperatures and desiccation by reducing 79 exchanges with a stressful environment (Alvarez et al., 2006; Massion, 1983). Thus 80 comparing populations from different climates reared in a common garden, low metabolic 81 rate genotypes should be observed in hot and dry environments when compared with cooler 82 and wet environments, according to the MCA hypothesis and to the hypothesis on resistance 83 to desiccation.

84 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 85 thus fewer resources for fitness traits such as fecundity. Consequently, a lower metabolic rate 86 is expected when resources are limited, to reduce the rate of resource consumption (Hoffmann 87 88 and Parsons, 1997; Mueller and Diamond, 2001). In parasitic wasps, lipids represent the main 89 energy resource allocated to survival, reproduction and dispersal (Ellers and van Alphen, 90 1997; Ellers et al., 1998; Rivero and Casas, 1999). These organisms were thought to be 91 unable to synthesize lipids during adult life (for a review, see Visser and Ellers 2008) but 92 Visser et al. (2010) recently described lipogenesis in some species. Moiroux et al. (2010) 93 found intraspecific variations in lipogenesis ability in adults, or at least in lipid accumulation, 94 between populations of a Drosophila parasitoid, Leptopilina boulardi (Hymenoptera: 95 Eucoilidae), originating from different climates. Females from an Iranian hot and dry area 96 were able to synthesize lipids during adult life whereas a strong decrease in lipid quantity was 97 observed in populations from the mild and humid Iranian coast of the Caspian Sea, suggesting 98 that no lipogenesis occurred there. Thus, the differences in the limitation in intrinsic resources 99 between populations from different climates may represent a difference in physiological 100 constraints that is likely to have affected the evolution of metabolic rate. Indeed, the cost of 101 maintaining a high metabolic rate on fitness-related traits should be stronger when resources 102 are limited.

103 The main goal of this study was precisely to investigate if metabolic rate has mainly evolved 104 in response to climate or was constrained by differences in intrinsic resource limitations. We 105 thus compared the  $CO_2$  production of females originating from a hot and dry climate and able 106 to synthesise lipids with females from a mild and humid climate where no lipid accumulation 107 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.

113

# 114 Material & Methods

115

### 116 Rearing

117 Four genetically distinct populations (Seyahooei 2010) of Leptopilina boulardi (Barbotin, 118 Carton & Keiner-Pillault, 1979), a solitary endoparasitoid that mainly attacks Drosophila 119 melanogaster and D. simulans larvae, were collected in July 2006 in Iran - two in a mild and 120 humid region and two in a hot and dry area - using twelve banana bait traps per population. 121 Each open trap (i. e. a plastic container with a 3 cm diameter hole covered with mesh with 2 122 mm openings) was colonised by five to twenty females and several hundreds of offspring 123 were produced in each of them. From the offspring thirty females per population were taken 124 to set up lab cultures. Two populations - Chalus and Seyakhal (called Mild humid 1 and 2 in 125 next sections) – originated from a humid mild environment, in the coastal plain of the Caspian 126 Sea. Dorcheh and Zamankhan strains (called Hot dry 1 and 2 in next sections) originated from 127 a very dry, hot area close to Esfahan, although the location of our traps was in the valley of a 128 snow-fed river that keeps water throughout most summers and allows fruit productions. 129 Climatic data is presented in Table 1.

130 *D. melanogaster* used as hosts in the cultures originating from strains collected in the 131 Netherlands in 1960. Parasitoids and fruit flies were reared at  $25 \pm 1$  °C -optimal rearing 132 temperature for *L. boulardi* considering the numbers of individuals that successfully emerged 133 as adults-,  $50 \pm 10$  % RH and 16L: 8D photoperiod.

For the experiment, *L. boulardi* females oviposited in separate jars for 12h at 25°C in 135 150 second instar *D. melanogaster* larvae laid in a two-hour period in a baker's yeast 136 suspension. The parasitized larvae were then separated into four groups which were placed at 137 20, 22.5, 25 and 27.5°C. The metabolic rate was measured at these four temperatures as 138 different reaction norms may have been selected in different climates. We also measured the 139 amount of lipids to check for the lipid accumulation during adult life in our populations with a

- 140 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 measuringmetabolic rate and the second one for measuring lipids.
- 143 No individuals from Dorcheh emerged at 20°C so no data is available at this temperature for
- 144 this population. It is likely that diapause occurs at this temperature as observed in other
- 145 populations of *L. boulardi* (Carton & Claret, 1982).
- 146

147 Metabolic rate. Routine metabolic trait -that corresponds to basal metabolic rate plus some 148 contribution from activity- was measured at the same temperature at which parasitoids were 149 reared (i.e. 20, 22.5, 25 or 27.5°C). We used flow-through respirometry to measure metabolic 150 rate of emerging female wasps. Fifteen to twenty samples per temperature were tested for 151 each population. To detect a strong signal, twenty individuals were pooled per sample. After having their fresh mass measured with a microbalance Sartorius (±0.001mg), fourteen 152 153 samples of twenty 1-d old individuals were placed in a climate room at rearing temperature in 154 separate small cylindrical chambers and their CO<sub>2</sub> production was measured with an infra-red 155 CO<sub>2</sub> analyser (LI-COR LI6251). A fifteenth chamber was kept empty as a control. A Sierra 156 mass flow controller maintained constant flow rates of dry, CO2-free air. Air was drawn off 157 from the environment and CO<sub>2</sub> and water were scrubbed with a Drierite-Ascarite column. 158 Four records of five minutes for each sample were performed with a gap of 90 minutes 159 between two recordings, and were automatically transformed by a program recorded in 160 DATACAN software (Sable Systems, Las Vegas), to convert the measure from ppm to µL-161 CO<sub>2</sub> per hour, taking into account the flow rate. The first recording allowed individuals to get 162 used to their new environment and was not considered for analysis. The value measured in the 163 control chamber was subtracted to the values measured in chambers with wasps. We 164 considered the average of the last three recordings as a measure of metabolic rate for each 165 sample. Recordings were performed at regular hour during photophase (10:00 am to 04:00 166 pm) and scotophase (6:00 pm to 12:00 pm) to cover different periods of the day. Routine 167 metabolic rate is generally measured during scotophase, when insects are inactive, however 168 we observed strong differences in locomotor activity among the four populations during this 169 period, females from the desert area being more active than females from the mild area 170 (Moiroux et al. 2010). During the photophase, we did not observe any significant difference 171 in the locomotor activity among populations. Measures of the metabolic rate were thus not 172 mainly affected by differences in locomotor activity during this period and we may mainly 173 thus consider differences as the result of differences in the basal metabolic rate.

174

175 Lipid analysis. The amount of lipids was investigated at three ages to detect lipid 176 accumulation during adult life. Adult parasitoids used for measures of lipid quantity were 177 reared at the same temperature than their larval development temperature, 12L: 12D in glass 178 jars on an Agar-Nipagine substrate, fed with acacia honey diluted to 20% and distributed ad 179 libitum. We measured the lipid content of 20 females per age and temperature for each 180 population, using a colorimetric analysis protocol developed for parasitoids by Giron et al. 181 (2002), adapted from van Handel (1985). Twenty 1-d, 5-d and 10-d old females were 182 individually placed in Eppendorf tubes, killed in liquid nitrogen and conserved at -80°C. 183 After defrosting, the length of the left hind tibia was measured ( $\pm 0.01$ mm) with the numeric 184 image analysis software Pegasus Pro V4 under a binocular (Olympus SZ-6045TR) linked to a 185 video camera (JVC KY-F). Then both ovaries were removed, as we were interested in stored 186 lipids, and wasps were individually placed in Eppendorf tubes in 50 µL of Ringer solution and 187 crushed with a plastic pestle in 300 µL of methanol. Tubes were centrifuged for 15 minutes at 188 4°C and 1400 rpm. 150 µL of chloroform and 60 µL of 2% sodium sulphate solution were 189 then added to samples and tubes were vortexed and stored at 4°C for one night. The next day, 190 tubes were centrifuged for 15 minutes at 1400 rpm. 300 µL of supernatant per sample were 191 transferred to other tubes that were put on an aluminium heat block at 90°C until total 192 evaporation was complete. 40 µL of 98% sulfuric acid were then added, tubes were heated 193 again at 90°C for two minutes and cooled down on ice. 960 µL of Vanillin Reagent were 194 added and tubes were vortexed and left for 15 minutes at ambient temperature. The 195 absorbance was read at 525 nm with a spectrophotometer (VersaMAX). The calibration curve 196 we used to transform absorbance into concentrations was made with standard sunflower oil. 197

## 198 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 analyze 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,209 2008).

210

# 211 **Results**

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 (Figure 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).

219 In every population, we observed a significant effect of rearing temperature on metabolic rate 220 and there were no differences between populations in the shape of metabolic rate reaction 221 norms (Figure 1). The elevation was indeed different but the slopes were similar. Metabolic 222 rate with light increased with temperature between 20°C, 22.5°C and 25°C and decreased 223 between 25°C and 27.5°C in every population (Mild humid 1: df = 3, 68, F = 5.105, p = 0.015, 224 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 225 dry 2: df = 3, 71, F = 3.45, p = 0.027). The same pattern was observed in darkness (Mild 226 humid 1: df = 3, 68, F = 1.92, p = 0.051, Mild humid 2: df = 3, 74, F = 2.93, p = 0.035, Hot 227 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 228 no difference occurred between 20°C and 22.5°C for the populations from the mild humid 229 area.

230

231 Lipid analysis. There was no evidence that females from the mild, humid environment 232 accumulated lipids but did in those from the desert area (Figure 2). We indeed observed a 233 significant decrease in lipid quantity between emergence, five days and ten days in females 234 from the mild humid region reared at 20°C, 22.5°C, 25°C and 27.5°C (Table 3). At 22.5°C 235 and 25°C, we observed a significant increase in lipid quantities in females from the hot and 236 dry environment between emergence and five days but no difference was observed between 237 five days and ten days. No significant difference was observed at 27.5°C in these females 238 between emergence, five days and ten 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 241 occurred at five (df = 7, 72, F = 5. 21, p = 0.01) and ten days (df = 70, F = 6.80, p = 0.014) of 242 adult life.

243

## 244 **Discussion**

245 We observed that females living in the hottest and driest area had the highest metabolic rate, 246 independently of rearing temperature or presence/absence of light, and thus of their locomotor 247 activity level (figure 1). Our results are in contradiction with the great majority of studies on 248 evolution of metabolic rate in response to climate where populations/species from temperate 249 climates were found to have a higher metabolic rate than the ones from hot and/or dry 250 climates (e.g. Addo-Bediako et al., 2002, Berrigan and Partridge, 1997). Authors suggested 251 that high metabolic rates have been selected in cold environments to compensate for the 252 negative effect of low temperatures on metabolism, or that low metabolic rates have been 253 selected in hot and dry climates to limit effects of desiccation and stressful temperature 254 (Massion, 1983). Contrary to these studies, our results suggest that geographic variations of 255 routine metabolic rates have probably not been mainly selected in response to climate of 256 origin in these parasitic wasps, but more likely in response to the limitation in intrinsic 257 resources.

258 Interestingly, we were able to show, as in a previous study (Moiroux et al., 2010), that 259 lipogenesis occurs in populations from the hot and dry area and is unlikely to occur in 260 populations from the mild humid area (Figure 2). When feeding on carbohydrate sources such 261 as nectar, females from the hot dry area produce lipids de novo at 22.5°C and 25°C whereas 262 females from the mild climate probably do not, or at least consume them faster than they 263 synthesize. At 20°C and 27.5°C, there was no clear evidence of lipogenesis in females from 264 the hot dry area as no difference in lipid content with age was found. However, the lack of 265 decrease with age in lipid content suggests that lipogenesis may also occur at these 266 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. 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 aslongevity and fecundity.

276 It is likely that lipogenesis ability has allowed the selection for a higher metabolic rate, by 277 affecting the energetic constrain of a trade-off between metabolic rate and fitness-related 278 traits, but it is probably not a factor that has selected for metabolic rate. Having a higher 279 metabolic rate has to be advantageous to be selected. In the desert area, parasitic wasps have 280 to cross large distances because laying opportunities are concentrated in distant orchards 281 distributed along a river. Moiroux et al. (2010) suggested that lipogenesis may have been 282 selected in populations from the hot and dry area as a large amount of lipids is required to fly 283 over large distances (Ellers et al., 1998). The present study advocates towards additional 284 evolutionary advantages for lipid accumulation. The decrease in resource limitation may have 285 changed physiological constraints there and allowed for the selection of a higher metabolic 286 rate in this region, disrupting the expected evolution of metabolic rate in response to climate. 287 Here, a high routine metabolic rate may be adaptive as it may allow wasps to rapidly produce 288 ATP (Kolluru et al., 2004) when performing energetically costly flights (Brett and Groves, 289 1979; Cano and Nicieza, 2006) to find hosts. The increased locomotory activity would 290 compensate for the long distances between patches of hosts in a dry area and could result in a 291 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)

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298 Reaction norm. Metabolic rate increased with temperature between 20°C and 22.5°C as 299 found in the great majority of studies on temperature-dependence of metabolism. However we 300 observed a decrease between 25°C and 27.5°C suggesting that this last temperature is 301 physiologically stressful and may negatively affect the metabolism and activity of organisms. 302 The strong decrease in the number of emergences between these two temperatures (from 80% 303 at 25°C in all populations to 50% at 27.5°C in the dry area and 30% in the mild area) confirms 304 that 27.5°C is a stressful temperature. Moiroux et al. (2010) found that females from the hot 305 dry area were more active at night. In this paper, authors suggested that this shift to nocturnal 306 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.

309 Even if we observed a higher metabolic rate in populations from the hot region than in 310 populations from the mild area, similar metabolic rate reaction norms were found in the four 311 populations. Our results hence suggest that no selection on reaction norms of metabolic rate 312 or on strength of phenotypic plasticity occurred. Metabolism obeys strict physical and 313 chemical principles governing the transformations of energy and materials, among which the 314 most relevant are the laws of mass and energy balance and thermodynamics (Brown et al., 315 2004). It is likely that no selection on the strength of phenotypic plasticity of metabolic rate 316 apply as that would probably involve selecting for changes in these laws.

317

318 **Conclusion.** The evolution of metabolic rate, a central element in evolution of life history 319 traits, has always been considered as the result of environmental factors, particularly 320 temperature and environmental resource limitations or unpredictability. We suggest that 321 differences in physiological constraints between populations may have affected this evolution 322 and should be integrated when investigating geographic variations of metabolic rate.

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**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 Organization). We considered the average of each parameter measured every day from April to September, which is the period of activity of *Leptopilina 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

<sup>478</sup> **Table 2.** Results of ANCOVA comparing metabolic rate of the four 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.

Temp (°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

**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.

	Populations				
Temp (°C)	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	
479					

- Figure 1. Metabolic rate during photophase (A) and scotophase (B) measured at four temperatures for the four populations. Error bars: ± s.e.. No individual from Dorcheh (hot dry 1) emerged at 20°C.
- **Figure 2.** Temporal variations in the amount of lipid for the four populations (n= 20 females/population). Error bars:  $\pm$  s.e. No individual from Dorcheh (hot dry 1) emerged at 20°C.