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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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13

14 Running headline: Lipogenesis affects the evolution of metabolic rate in parasitoids.

15

16

17 **Abstract.**

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

25 We compared CO<sub>2</sub> production of females from four populations of a *Drosophila* parasitoid,  
26 *Leptopilina boulardi*, as an estimate of their metabolic rate. Two populations from a hot and  
27 dry area able to synthesize lipids *de novo* at adult stage were compared with two populations  
28 originating from a mild and humid climate where no lipid accumulation during adult life was  
29 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  
36 integrated when investigating geographical variations in metabolism as this factor may disrupt  
37 evolution in response to climate.

38

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

40

## 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  
44 into the environment (Brown et al. 2004). All of these processes define metabolism.  
45 Metabolic rate (i.e. the rate of energy uptake, transformation and allocation), generally  
46 measured as O<sub>2</sub> consumption or CO<sub>2</sub> 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  
63 Metabolic Compensation hypothesis (Conover and Schultz, 1995). The MCA hypothesis  
64 assumes that high metabolic rates have been selected in cold environments to compensate for  
65 the negative effect of low temperatures on metabolism. This would result in higher metabolic  
66 rates in populations or species from cold climates when compared with warm-adapted  
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  
69 in favour of MCA in fish (Álvarez et al., 2006; Cano and Nicieza, 2006), grasshoppers  
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)

74 provided a strong evidence for the MCA hypothesis in a global-scale analysis on interspecific  
75 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.  
85 Indeed, a higher metabolic rate involves a higher amount of energy to maintain the body and  
86 thus fewer resources for fitness traits such as fecundity. Consequently, a lower metabolic rate  
87 is expected when resources are limited, to reduce the rate of resource consumption (Hoffmann  
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<sub>2</sub> 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

108 be expected (1) if metabolic rate has been mainly selected in response to climate (MCA  
109 hypothesis and hypothesis on resistance to desiccation), we should observe a lower metabolic  
110 rate in populations from the hot and dry area (2) if the evolution of metabolic rate was  
111 differently constrained by differences in resource limitation, we may observe a higher  
112 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.

134 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).  
141 When emerging, wasps were separated into two groups. The first one was used for measuring  
142 metabolic 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. boulandi* (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  
152 having their fresh mass measured with a microbalance Sartorius ( $\pm 0.001$ mg), fourteen  
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, CO<sub>2</sub>-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  $\mu$ L-  
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  $-80^{\circ}\text{C}$ .  
183 After defrosting, the length of the left hind tibia was measured ( $\pm 0.01\text{mm}$ ) 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  $\mu\text{L}$  of Ringer solution and  
187 crushed with a plastic pestle in 300  $\mu\text{L}$  of methanol. Tubes were centrifuged for 15 minutes at  
188  $4^{\circ}\text{C}$  and 1400 rpm. 150  $\mu\text{L}$  of chloroform and 60  $\mu\text{L}$  of 2% sodium sulphate solution were  
189 then added to samples and tubes were vortexed and stored at  $4^{\circ}\text{C}$  for one night. The next day,  
190 tubes were centrifuged for 15 minutes at 1400 rpm. 300  $\mu\text{L}$  of supernatant per sample were  
191 transferred to other tubes that were put on an aluminium heat block at  $90^{\circ}\text{C}$  until total  
192 evaporation was complete. 40  $\mu\text{L}$  of 98% sulfuric acid were then added, tubes were heated  
193 again at  $90^{\circ}\text{C}$  for two minutes and cooled down on ice. 960  $\mu\text{L}$  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**

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

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

207



208 All analyses were carried out using R software version 2.8.1 (R Development Core Team,  
209 2008).

210

## 211 **Results**

212 **Metabolic rate.** The sampling site had a significant effect on metabolic rate. Populations  
213 from the desert area generally had a higher metabolic rate than those from the mild, humid  
214 climate at every temperature, as well as at different times in the photoperiod (Figure 1). Hot  
215 dry 1 and 2 populations had a higher metabolic rate than Mild humid 1 and 2 populations with  
216 fresh mass as covariate at 22.5°C, 25°C and 27.5°C during photophase and scotophase (Table  
217 2). Hot dry 2 population had a higher metabolic rate than mild humid 1 and 2 populations at  
218 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).

239 Females from the mild and humid area emerged with more lipids than females from the hot  
240 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.

267 The consequence of these differences is that limitation in intrinsic resources is stronger in  
268 females from the mild climate even if, as observed in this study, they emerge with more lipids  
269 than females from the hot and dry region. This increase in metabolic rate occurs without  
270 shortening lifespan or decreasing egg load, as females from the dry area lived longer and  
271 produced more eggs than females from the mild area (Moiroux et al., 2010). It is likely that a  
272 higher metabolic rate may have not been selected in a hot and dry climate without lipogenesis  
273 ability, as the cost associated with a higher amount of energy to maintain organism would

274 have strongly decrease the amount of energy available for fitness-related traits such as  
275 longevity 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 Nieceza, 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.

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

297

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.

307 Results on metabolic rate at 27.5°C tend to confirm that avoidance of high temperatures may  
308 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.

323

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332

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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.

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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

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**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.

Temp (°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

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481 **Figure 1.** Metabolic rate during photophase (A) and scotophase (B) measured at four  
482 temperatures for the four populations. Error bars:  $\pm$  s.e.. No individual from Dorcheh  
(hot dry 1) emerged at 20°C.

483 **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.