Differentiation of thermal reaction norms between marginal and core populations of a northward expanding parasitoid

Running title: Geographic thermal reaction norms

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Abstract

Understanding the speed of and the type of mechanisms that species use to adapt to rapid change is a central question in evolutionary biology. Classically, the two mechanisms denoted in the literature that allow individuals to address these environmental changes are either phenotypic plasticity or rapid evolutionary changes. However, phenotypic plasticity itself can evolve rapidly. In this study, we investigated the genetic differentiation between marginal and core populations of a high-trophic level insect, *Leptopilina boulardi*, a *Drosophila* parasitoid, which has exhibited a very rapid progression northward of its geographical range. Several life history traits have been investigated in different populations according to four fluctuating thermal regimes that mimic the thermal conditions in the field. We found that at low developmental temperature, the two northern marginal populations that have to face a colder winter, survive longer than the two core populations. In addition, the northernmost populations exhibit a higher potential fecundity, a higher starvation resistance and a larger amount of energy at low temperatures. These significant genetic differentiations with genotype-by-environment interactions show that a rapid genetic differentiation of the shape of thermal reaction norms is possible when populations have to cope with new environments.

Key words: Phenotypic plasticity, life history traits, fluctuating temperature, climate change, range expansion.

Introduction

In recent years, important literature has emerged on the mechanisms of adaptation of species to environmental changes in relation to human activities, be it the impact of climate change or the increase of biological invasions (Chown et al. 2007, 2015; Ghalambor et al. 2007; Hulme

2008; Prentis et al. 2008; Engel et al. 2011; Hoffmann and Sgrò 2011; Bock et al. 2015; Sgrò et al. 2016; Estoup et al. 2016; Gibert et al. 2016). The questions addressed in all these studies are how quickly and by what mechanisms species can adapt to a rapid change of the environment that can generate strong new selective pressures. There are typically three non-mutually exclusive scenarios in the literature by which animals may respond to these changes: i) natural populations can migrate to more favorable geographical areas (e.g. (Parmesan and Yohe 2003; Root et al. 2003; Chen et al. 2011), or remain and adapt to the new conditions ii) through phenotypic plasticity (e.g., Richards et al. 2006; Charmantier et al. 2008; Nicotra et al. 2010; Drown et al. 2011) or iii) by selection for genotypes adapted to the new environmental conditions (evolution) (e.g., (Hoffmann and Sgrò 2011; Urbanski et al. 2012; Hamilton et al. 2015). Plastic responses, i.e. the ability of a genotype to express a different phenotype in various environments, are generally considered to allow rapid responses in a single generation, while evolutionary responses are slower because they require changes in the genetic make-up of populations. However, these two scenarios are not exclusive since plasticity by itself can evolve ((Gotthard et al. 1995; Ghalambor et al. 2007; Scoville and Pfrender 2010) and thus can show greater or lesser variability between populations of the same species (e.g., (Delpuech et al. 1995; Morin et al. 1999; Ayrinhac et al. 2004; Ris et al. 2004; Lind and Johansson 2011; Frei et al. 2014; Pereira et al. 2018). Theoretical studies have suggested that for species continuously distributed in space along an environmental gradient, greater plasticity is expected in marginal populations (Chevin and Lande 2011). How rapidly such an evolution can occur is difficult to assess empirically and requires a situation with a recent and well described range expansion. A comparison of phenotypic plasticity between populations from the core and the marginal recently colonized should allow estimation of this rate of evolution of phenotypic plasticity. In plants that can be easily cloned and that are directly affected by environmental variations because of their inability to move, this type of study comparing geographic and altitudinal

ecotypes exists (e.g. (Nicotra et al. 2010). In insects, except for some iconic (e.g. butterflies) or harmful (e.g. mosquitoes) species, long-term monitoring is rare. Moreover, studying phenotypic plasticity requires the possibility of conducting laboratory experiments that control the environmental variation, a situation that is not possible in all organisms.

Leptopilina boulardi (Hymenoptera: Eucoilidae) is a larval endoparasitoid that uses *Drosophila* as a host, mainly *D. melanogaster* and *D. simulans*, to achieve its larval development until it is a free-living adult. The geographical distribution of *L. boulardi* ranges from tropical to subtropical areas in Africa and America, including areas with a Mediterranean climate (Hertlein 1986; Allemand et al. 2002). In Europe, *L. boulardi* has been reported all around the Mediterranean basin in Spain, southern France, North Africa and the Middle East ((Barbotin et al. 1979; Nordlander 1980; Allemand et al. 2002; Fleury et al. 2009). (Delava et al. 2014) showed that in the southeast of France, *L. boulardi* is moving very rapidly northwards, with an average rate of range expansion of 170 km in 19 years (1993-2011), exceeding previously observed rates for small insects. This range expansion is not limited by host availability and could be related to climate warming since this area was characterized by an annual warming of temperature with a northward displacement of isoclimatic lines by approximately 100-130 km over 33 years (Lelièvre et al. 2011). However, during the same period, winters have become colder, with an average temperature decrease of -1.58°C that may imply the adaptation of the species to new local conditions.

In this paper we study how and how quickly latitudinal temperature variations can change the shape of reaction norms. We thus compared thermal reaction norms of various life history traits between four populations, two populations 150 km apart located in the central area of *L. boulardi* and two populations about 20 km apart located in the recently colonized northern area of *L. boulardi* (marginal populations) range. We expect a genetic differentiation of

fitness-related traits between populations from marginal and core areas in response to these different thermal selective pressures, with possible genotype-by-environment interactions.

Materials and methods

BIOLOGICAL MODEL, COLLECTION SITES AND LABORATORY REARING

Leptopilina boulardi is a solitary (only one offspring can survive per host whatever the number of eggs deposited) endoparasitoids that attacks first and second stages of Drosophila larvae (Carton et al. 1986). In many cases, the issue of parasitism is the emergence of a parasitoid but there can also be a precocious parasite death induced by the immune response of the host (called encapsulation) leading to the emergence of a Drosophila. In some cases, also, a physiological inadequacy can lead to the death of both partners (Fleury et al. 2009). In *L. boulardi*, females can be infected by a maternally transmitted DNA virus known as LbFV (Leptopilina boulardi filamentous virus), frequently leading to a situation of superparasitism (deposition of supernumerary eggs into previously parasitized larvae) (Varaldi et al. 2003). Previous work showed Lbfv has no detectable effect on female survival, but has a slightly positive effect on egg load and a weakly negative effect on tibia length and developmental rate (Varaldi et al. 2005).

Parasitoid populations were collected from orchards using several banana bait traps (at least 7 traps per population) placed in four sampling localities in southeastern France (Supplementary S1). Two sampling sites, Eyguières (S1, Latitude 43°41'N) and Gotheron (S2, Latitude 44°58'N), were located in the south of the Rhône-Saône Valley, where a Mediterranean climate prevails and where *L. boulardi* has been well established for many years (core area). The two other localities, Sainte-Foy-lès-Lyon (N1, Latitude 45°44'N) and Saint-Maurice-de-Beynost (N2, Latitude 45°49'N), were located in area where *L. boulardi* was absent in the 1990s, but that was progressively colonized by this species during the last

117 twenty years (marginal area) (Delava et al. 2014). The most southern central population and
118 the most northern marginal population are separated by approximately 240 km.
119 Parasitoids sampled in the 4 localities were used to established 4 mass populations of *L.*
120 *boulardi* that were reared on a standard laboratory strain (more than 250 generations in the
121 lab) of *Drosophila melanogaster* in a controlled environment at 25 ± 0.3°C, 70% RH (Sanyo
122 MLR-351H climatic chamber), with a photoperiod of 14L:10D. The experiments were
123 conducted after approximately 10 generations in the laboratory.
124
125 **TEMPERATURE TREATMENTS DURING DEVELOPMENT**

126 *L. boulardi* shows a thermal specialization with an optimum around 25 °C, and a fall of its
127 performance when the temperature deviates by more than 2 °C from this optimum (Fleury et
128 al. 2009; Moiroux et al. 2013). It is an over-wintering species with a facultative larval
129 diapause, almost 100% of the larvae entered into diapause at 17.5 °C (Claret and Carton
130 1980). Because of this diapause, developmental plasticity has never been investigated in this
131 species, but it has been recently shown that the use of a fluctuating thermal regime allows the
132 larval diapause to be overcome (Delava et al. 2016). In this paper, we have thus simulated the
133 daily temperature fluctuation as closely as possible to the natural thermal regime. Towards
134 that aim, temperature was recorded every hour (EasyLog USB data logger) in seven localities
135 of the Rhône-Saône valley from latitude 44°58'N to latitude 46°24'N over 62 days (from July
136 1 to August 31, 2010), when the abundance of *L. boulardi* is high. For each hour of the day,
137 the median of the 62 days was calculated and used to create a fluctuating thermal regime that
138 showed an average temperature of 22.7°C. This thermal regime was then modified by
139 increasing the temperatures by + 1.5°C, - 1.5°C and - 3°C to obtain 4 fluctuating thermal
140 regimes with the same amplitudes but with different average temperature: 19.7°C, 21.2°C,

22.7°C and 24.2°C (Supplementary S2). In the following text, fluctuating thermal regimes will be named using these average values.

The experiment was conducted simultaneously in 4 identical climatic chambers (Sanyo MLR-351H) in which the temperature was controlled according to the tested thermal regime with a precision of 0.1°C. In the 4 chambers, the temperature varied every two hours, while the photoperiod and humidity were the same (12L: 12D, 70% RH), and the temperature was recorded with a datalogger (EasyLog USB).

EXPERIMENTAL DESIGN

One hundred twenty-five *D. melanogaster* eggs were deposited into rearing vials (15 replicates per thermal regime and per population, 240 vials in total) (see Fig. 1). Vials were then randomly assigned to the 4 incubators (60 vials per incubator in total). After 24 h (to allow eggs to hatch), a single female parasitoid was introduced into each vial and removed 24 h later.

All females were kept to check the presence of LbFV viral particles by PCR using the protocol described by (Patot et al. 2009). We confirmed previous results found in (Patot et al. 2010), i.e. no parasitoid from N1 and N2 (populations from marginal region) were infected by the virus LbFV, while most parasitoids from S2 and S1 (populations from core region) were infected by LbFV (78% at S2 and 94% at S1 were infected).

For each thermal regime, five control vials (20 in total) with only the 125 *D. melanogaster* eggs but without *L. boulardi* were used to assess the quality of the development of *Drosophila* larvae in the absence of parasitoids.

All vials were maintained under the 4 fluctuating thermal regimes until the insect's complete development. Adult females used to measure adult life history traits, such as lifespan and fecundity, were kept under the same thermal conditions as larval development.

166

167 **TRAITS RELATED TO THE DEVELOPMENT OF THE PARASITOID**

168 The insects' emergence was checked daily, and all adults (*Drosophila* and then parasitoids)
169 were counted in each vial. To estimate the host immune response, adult *Drosophila* were
170 dissected under a microscope by crushing the entire individual between two glass slides, and
171 the number of flies containing encapsulated parasitoids egg (the immune response of
172 *Drosophila* host) was recorded. Since the number of capsules was negligible (less than 1%)
173 and was not significantly different by the thermal regimes (GLM, $\chi^2_{(3,221)} = 335$, $P = 0.47$) and
174 populations ($\chi^2_{(3,218)} = 324$, $P = 0.15$), we did not include this parameter in further estimates of
175 parasitoid development.

176 Using the number of emerging Drosophila and parasitoids counted daily in each vial, two
177 parameters related to the host-parasitoid interaction were calculated.

178 ***- Host infestation rate (IR).*** This parameter measures the number of *Drosophila* parasitized
179 by wasps no matter the issue of parasitoids development. IR was calculated as $IR_i = (nc - n_{di})$
180 / nc, where nc is the average number of *Drosophila* emerging from control vials and n_{di} is the
181 number of *Drosophila* that emerged from each test vial.

182 ***- Success of parasitism (SP).*** This trait estimates the percentage of parasitized hosts that give
183 rise to adult parasitoids (larval survivorship of the wasps). SP was calculated for each
184 female's offspring as follows: $SP_i = n_{pi} / (125 - (n_{di} / s))$, where n_{pi} is the total number of
185 parasitoids produced in each vial, 125 is the total number of *Drosophila* hosts eggs in each
186 vial, n_{di} is the total number of adult flies emerging from each vial (and thus that escaped from
187 parasitism) and s is the survival rate of flies emerging from control vials ($s = nc / 125$).

188 We also measured the ***Parasitoid development time***, i.e., the development duration of the
189 parasitoid from the day of deposit of *Drosophila* eggs in vials to the emergence of an adult
190 parasitoid.

191

192 **ADULT PARASITOID PHENOTYPIC TRAITS**

193 ***- Potential fecundity***. Egg-load was measured on 5-day-old honey-fed females (20 per
194 population and per developmental thermal regime, 320 in total). Parasitoid females were
195 individually dissected in a drop of Ringer's solution under a binocular microscope (BBT
196 Krauss). One of the two ovaries was placed under a microscope (Axio Imager, AxioCam,
197 Software Axiovision LE; Zeiss, Thornwood, NY, USA) and was photographed. The eggs
198 were counted on each photograph using ImageJ. The total fecundity of the individual was
199 estimated as being twice the number of eggs contained in one ovary.

200 ***-Size*** The size of females tested for fecundity was determined by measuring the length of their
201 right tibia using the same microscope. Tibia length was shown to be a good proxy of
202 individual size in parasitoids (Cronin and Strong 1996; Nicol et al. 1999), and individual size
203 is positively correlated with most life-history traits (Godfray 1994).

204 ***- Starvation resistance.*** After emergence, some parasitoid females were placed in vials
205 without a host and food but with sterile moistened cotton to measured their lifespan (10
206 females per vial and 4 vials per population and per thermal regime, 640 females in total).
207 Cotton was moistened daily to provide humidity. Dead parasitoids were counted twice a day,
208 every morning and evening (at 9:00 a.m. and 6:00 p.m.).

209

210 **ADULT PARASITOID ENERGETIC RESOURCES**

211 A part of some newly emerged females was stored at -20°C to measure their energetic
212 reserves. Proteins, lipids, sugars and glycogen content were measured according to the
213 protocol of (Foray et al. 2012). Since *L. boulardi* is a small insect (approximately 2 mm),
214 preliminary analyses were conducted to test the sensitivity of the method for all metabolic
215 compartments. On the basis of this preliminary test, we decided to pool 8 individuals to
216 perform extraction on 10 repetitions per population and per thermal regime (160 samples in

total). The value obtained in each energy compartment for a pool of 8 individuals was divided by 8 to obtain an average amount per individual. Because the quantities of energy reserves are correlated with the individual's size, we divided all quantities (for each compartment) by the average size measured from 20 females per population and thermal regime to take into account this size effect. Then, the total energy amount in J/mg available at emergence was assessed using the following conversion factors: 16.74 J/mg carbohydrates and proteins and 37.65 J/mg lipids (Rivero and Casas 1999). Finally, the amounts of proteins, lipids, sugars and glycogen (in joules) were divided by the total amount of energy in order to obtain the percentage of each compartment. Because the amount of free sugars and glycogen was very low (2.5% and 8.0%, respectively), they are not presented in this study.

STATISTICAL ANALYSES

All analyses were performed with the R software (version 2.14.1) (R Development Core Team, (2011). Vials with no parasitoid emergence were excluded from the analysis. Except for the number of capsules and adult survival, all life history traits were analyzed using linear models after adequate transformation to comply with the assumptions of normality and homoscedasticity when necessary (log transformation for 1-IR and arcsine square-root transformation for SP). A generalized linear model was used to analyze the number of capsules with a quasi-Poisson error (log link function) to take into account the over-dispersion of the trait. Survival was analyzed by means of a Weibull distribution using the function "survreg" from the R package "survival". We included vials as a random effect using the "frailty" function, but since it did not improve the model, this effect was removed. In all models, the population (qualitative variables) and the developmental temperature regime (quantitative variable) were tested as fixed effects. We have also included a quadratic or cubic effect of developmental temperature to better fit the shape of the performance curve when

appropriate. Initial models were simplified by stepwise regression while minimizing Akaike's information criterion (AIC). The results include only the best models selected by the lowest AIC criterion (Johnson and Omland 2004).

A linear model was also performed to check whether the potential fecundity was correlated with body size (estimated by the length of the tibia). Since correlations between these two variables were found non-significant, the size was not included in the model explaining fecundity.

For all models, comparisons between populations and thermal regimes were then possible by changing the reference population and thermal regime in the contrast matrix.

Results

TRAITS RELATED TO THE DEVELOPMENT OF THE PARASITOID

Host infestation Rate (IR) and Success of Parasitism (SP)

No significant differences were observed for IR, no matter the developmental thermal regime or the population. SP exhibited a significant interaction between the population effect and the cubic effect of developmental thermal regime ($F = 5.23$, $P = 0.0018$) (Fig. 2A), with a lower SP value for the population of S2 at 24.2°C.

Development time

Development time significantly varied with both developmental thermal regime and populations, but no significant interaction was observed. Development time decreased when the average temperature of the developmental thermal regime increased ($F = 7867.28$, $P < 0.001$) (Fig. 2B) as classically observed for insect species. The development duration for the complete eggs to adult development was approximately 24 days at 24.2°C and increased until 34 days at 19.7°C. Development time also differed significantly between populations ($F = 27.10$, $P < 0.001$). The southern population, S1, developed significantly slower than the three

other populations (28.87 days±3.84 for S1 versus 27.91 days on average for the three other populations).

ADULT PARASITOID LIFE-HISTORY TRAITS

- Potential fecundity. For the four studied populations, female egg-load showed no significant differences between the developmental thermal regimes ($F = 0.0034$, $P = 0.95$). We thus pooled the data of these thermal regimes and compared them between populations. A strong population effect was observed ($F = 21.62$, $P < 0.001$), with females from the northern population, N2, showing a significantly higher egg-load (158 ±21.9) than the three other populations (N1, S2 and S1) (133 ± 30.7 on average) (Supplementary S3).

-Size. A significant effect of both population and developmental thermal regime was found on size, with no significant interaction. Surprisingly, and contrary to what is generally found in insects, size estimated by the tibia length significantly increased with developmental temperature ($F = 7.41$, $P = 0.0017$) (Fig. 3A). We also found that females from the southern population, S1, were significantly bigger (301.61 ±9.94 μm) than those from N2 and S2 (297.62 ±11.47 μm and 294.77 ±11.29 μm respectively).

- Starvation resistance. A significant interaction between developmental thermal regime and population (Dev = 9.44, $P = 0.024$) was observed for adult survival. As it is observed for many insects, we observed a decrease of survival when temperature increases for all populations (Dev = 414.13, $P < 0.001$). However, we found that at the lower thermal regime (19.7°C and 21.2°C), parasitoids from the northern marginal region (N2 and N1) lived significantly longer than parasitoids from southern populations (S2 and S1), whereas at 24.2°C no significant differences were observed between the four populations (Fig. 3B).

ADULT PARASITOID ENERGETIC RESOURCES

292 The developmental thermal regime significantly influenced the total amount of energy,
293 which decreased when temperatures become higher ($F = 75.99$, $P < 0.001$), with a significant
294 quadratic and cubic effect ($F = 12.44$, $P < 0.001$ and $F = 15.53$, $P < 0.001$ respectively).
295 Females developed at the two lower temperatures of 19.7°C and 21.2°C had significantly
296 more energy than females developed at 22.7°C and 24.2°C (Fig. 4). In addition, there was a
297 significant difference in the total amount of energy between populations ($F = 5.57$, $P =$
298 0.0012). Females from N2 had more energy than females from the three other locations,
299 especially at 19.7°C and 21.2°C, even if the interaction between population and
300 developmental thermal regime was not significant (interaction with linear effect, $F = 1.83$, P
301 $= 0.14$; interaction with quadratic effect, $F = 2.00$, $P = 0.12$; interaction with cubic effect, $F =$
302 2.63, $P = 0.053$).
303 Whereas no developmental thermal regime by population interaction was detected for the
304 total amount of energy, a genotype-by-environment interaction was significant for both
305 protein and lipid rates, but with an opposite trend. For the protein rate, a highly significant
306 developmental thermal regime by population interaction ($F = 22.38$, $P < 0.001$) resulted in a
307 lower protein content in northern marginal populations (N2 and N1) at higher temperatures,
308 whereas all populations were similar at low temperatures (Fig. 5A). In contrast, for the lipid
309 rate, the significant interaction between the variables population and developmental thermal
310 regime ($F = 13.48$, $P < 0.001$) was due to a higher rate for northern marginal populations at
311 the highest temperature of 24.2°C, whereas small differences among populations were
312 observed at low temperatures (Fig. 5B).
313

314 ## *Discussion*

315 The objective of this paper was to describe the reaction norms of an insect whose range has
316 recently been extended in relation to global warming. Several traits were thus studied under

four different thermal regimes and for four of the central and marginal range populations. The use of fluctuating temperatures allowed us to circumvent the parasitoid diapause (Delava et al. 2016), which is induced by relatively high temperatures, since 50% of parasitoids enter into diapause under a constant 20°C (Claret and Carton 1980). Our study thus constitutes the first investigation of the phenotypic plasticity of *L. boulardi* under this range of temperatures.

DIFFERENTIATION OF THERMAL REACTION NORMS OF LIFE HISTORY TRAITS

We found a significant differentiation between populations for various traits. The southern population, S1, develops faster and is bigger than the other populations, while the northern population, N2, has the highest fecundity.

According to the temperature-size rule, the typical pattern in ectotherms is that body size is negatively correlated with developmental temperature (Atkinson 1994; Angilletta and Dunham 2003). Our results depart from this rule since we found that the tibia length (the proxy of body size) regularly increases when the fluctuating developmental thermal regime increases. To our knowledge, this is the first time that such a result is highlighted in ectotherms. Another intriguing result is observed for potential fecundity, which is not affected by fluctuating developmental thermal regime, in contrast to the concave reaction norms that are generally observed using constant temperatures in insects (e.g., (Delpuech et al. 1995) on *Drosophila melanogaster* or (Ris et al. 2004) on *Leptopilina heterotoma*). Our results suggest therefore that using fluctuating temperatures can buffer the impact of developmental temperature at least for these traits and questions the generalization of the results using only constant temperatures.

A significant population-by-temperature interaction reflecting a modification of the shape of the thermal reaction norms between populations is found for two traits only: the success of parasitism and starvation resistance. Clearly, starvation resistance is the trait for which this effect is the most interesting. Indeed, for the two coldest thermal regimes (fluctuating

342 developmental thermal regime of 19.7°C and 21.2°C), survival is significantly higher for the
343 two marginal northern populations (N2 and N1) than for the core populations (S2 and S1),
344 while in the warmer regime, 24.2°C, there is no difference between populations. Since in the
345 field, the maximal and minimal temperatures are lower in the marginal than in the core region
346 (Delava et al. 2014), our results suggest a local adaptation of marginal populations that
347 survive better at low temperatures than at higher temperatures compared to the core
348 populations.
349 If the thermal gradient of the Rhone valley alone were responsible for the differences in
350 reaction norms between populations, one would expect these differences to be correlated with
351 the geographical distance between the populations. However, we observe that for the two
352 populations in the core area, which are very far apart, the shape of the reaction norms is
353 similar and very different from the shape of the reaction norms of the two populations in the
354 marginal area. Instead, our results suggest an adaptation of the populations to particular
355 environmental conditions in the marginal zone. In this northern zone, winter remains very
356 cold and an average temperature increase was only observed in spring and fall (Delava et al.
357 2014). The observed change in the shape of the response norms for starvation could therefore
358 be related to the ability of populations to persist in the marginal zone during the cold season.
359
360 **ENERGETIC CONTENT ANALYSIS**
361 The overall energy content decreased with the fluctuating developmental thermal regime
362 and varied among populations. In fact, the northern population (N2) had significantly more
363 energy than the three other populations especially at the lowest developmental temperature. In
364 parasitoids, the nutritional resources are provided by the host and are stored by the parasitoid
365 during ontogeny to constitute the only energy reserves available at emergence (Rivero and
366 Casas 1999; Pelosse et al. 2007). *L. boulardi* is a pro-ovigenic parasitoid (its egg stock is
367 complete and mature at emergence), which means that the total energy at emergence will be

368 exclusively allocated to maintenance and locomotion. Thus, the greater amount of energy of
369 N2 at 19.7°C can probably explain its better starvation resistance in a cold environment.
370 Because free sugars and glycogen represented negligible amounts of energy compared to
371 proteins and lipids, we have focused on these two latter energy compartments. A significant
372 population-by-temperature interaction was found for these two traits, with similar values
373 between populations in the low fluctuating developmental thermal regime but a significant
374 differentiation between populations in the high fluctuating developmental thermal regimes.
375 However, opposite tendencies were observed since the marginal populations (N2 and N1)
376 have a lower protein content but a higher lipid content than the core populations (S2 and S1).
377 These high fluctuating developmental thermal regimes were characterized by temperatures
378 above 30°C, which should constitute a particularly stressful environment, especially for the
379 marginal northern populations. One of the specific traits of the parasitoids is that they are
380 unable to synthesize lipids during adult life (Visser and Ellers 2008); as a consequence, the fat
381 reserves accumulated during development should presumably provide a significant advantage
382 for resisting extreme environments.
383
384 **Possible confounding effect with LbFV**
385 In the present study, we also confirm the initial observations of (Patot et al. 2010) that
386 populations in the central region (S2 and S1) are infected with LbFV, while those newly
387 established in the northern areas (N2 and N1) are free of infection. LbFV is known to
388 influence the life history traits of *L. boulardi* (Varaldi et al. 2005), and this effect is
389 potentially confounded by geographic variation in populations. However, LbFV infection is
390 known to cause a slowing of host development (Varaldi et al. 2005), yet we observe
391 significantly shorter development for the two populations in the marginal region (N2 and N1
392 uninfected) compared to that of S1 (the southernmost population in cline, almost entirely

393 infected with LbFV). Similarly, for fecundity, infected individuals are supposed to have a
394 higher egg number than uninfected individuals (Varaldi et al. 2005) whereas it is the N2
395 population (the most northern population of cline, uninfected by LbFV) that exhibited the
396 highest potential fecundity, reinforcing the hypothesis of a local adaptation of this population.
397 These results suggest that even if we cannot totally exclude an effect of the presence of LbFV
398 on the life history traits of the studied populations, this effect is not sufficient to hide the
399 genetic differentiation between populations.
400

401 ## *Conclusion*

402 Newly established marginal populations of *L. boulardi* significantly differed from core
403 populations for several life history traits. The northern population (N2) is particularly well
404 differentiated, with a higher starvation resistance, a higher potential fecundity and overall a
405 higher energy content. In this population, *L. boulardi* was observed for the first time five
406 years before we sampled the individuals for this study, showing that a rapid differentiation of
407 thermal reaction norms is possible and thus that the evolution of phenotypic plasticity can be
408 fast. This differentiation could be the result of natural selection but also due to random genetic
409 drift that can be frequent in marginal populations often characterized by small size. In this
410 area, it has been shown that an increase of temperature in spring and autumn probably
411 allowed the northward displacement of *L. boulardi* but also that the coldest temperature
412 during winter could constitute an important selective pressure that could explain the local
413 adaptation of populations. To study the genetic structure of populations, the intensity of gene
414 flows between populations and the dispersive capacity of *L. boulardi* more finely,
415 investigations of population genetics using neutral molecular markers (e.g., RadSeq) are
416 required.
417

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References

Allemand, R., C. Lemaitre, F. Frey, M. Boulétreau, F. Vavre, G. Nordlander, J. Van Alphen, and Y. Carton. 2002. Phylogeny of six African Leptopilina species (Hymenoptera: Cynipoidea, Figitidae) parasitoids of Drosophila spp. with descriptions of three new species. Ann. la Société Entomol. Fr. 38:319–332.

Angilletta, M., and A. Dunham. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Am. Nat. 162:332–342.

Atkinson, D. 1994. Temperature and organism size - a biological law for ectotherms ? Adv. Ecol. Res. 25:1–58.

Ayrinhac, A., V. Debat, P. Gibert, A.-G. Kister, H. Legout, B. Moreteau, R. Vergilino, and J. R. David. 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Funct. Ecol. 18:700–706.

Barbotin, F., Y. Carton, and S. Kelner-Pillault. 1979. Morphologie et biologie de *Cothonaspis boulardi* n. sp. parasite de drosophiles. Bull. Soc. Entomol. Fr. 84:20–26.

Bock, D. G., C. Caseys, R. D. Cousens, M. A. Hahn, S. M. Heredia, S. Hübner, K. G. Turner,

442 K. D. Whitney, and L. H. Rieseberg. 2015. What we still don't know about invasion
443 genetics. Mol. Ecol. 24:2277–2297.
444 Carton, Y., M. Boulétreau, J. J. J. J. M. van Alphen, and J. C. van Lenteren. 1986. The
445 Drosophila parasitic wasps. Pp. 347–389 *in* C. T. Ashburner, ed. The Genetics and
446 Biology of Drosophila. Academic Press, London.
447 Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon.
448 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird
449 population. Science 320:800–3.
450 Chen, I.-C., J. K. Hill, R. Ohlemuller, D. B. Roy, and C. D. Thomas. 2011. Rapid Range
451 Shifts of Species Associated with High Levels of Climate Warming. Science (80-.).
452 333:1024–1026.
453 Chevin, L., and R. Lande. 2011. Adaptation to marginal habitats by evolution of increased
454 phenotypic plasticity. J. Evol. Biol. 24:1462–1476.
455 Chown, S. L., K. A. Hodgins, P. C. Griffin, J. G. Oakeshott, M. Byrne, and A. A. Hoffmann.
456 2015. Biological invasions, climate change and genomics. Evol. Appl. 8:23–46.
457 Chown, S. L., S. Slabber, M. McGeouch, C. Janion, H. P. Leinaas, M. A. McGeoch, C.
458 Janion, H. P. Leinaas, M. McGeouch, C. Janion, and H. P. Leinaas. 2007. Phenotypic
459 plasticity mediates climate change responses among invasive and indigenous arthropods.
460 Proc. Biol. Sci. 274:2531–2537.
461 Claret, J., and Y. Carton. 1980. Diapause in a tropical species, *Cothonaspis boulardi*
462 (parasitic Hymenoptera). Oecologia 45:32–34.
463 Cronin, J. T., and D. R. Strong. 1996. Genetics of oviposition success of a thelytokous
464 fairyfly parasitoid, *Anagrus delicatus*. Heredity (Edinb). 76:43–54. London: Oliver and
465 Boyd, 1947-.
466 Delava, E., R. Allemand, L. Léger, F. Fleury, and P. Gibert. 2014. The rapid northward shift

of the range margin of a Mediterranean parasitoid insect (Hymenoptera) associated with regional climate warming. J. Biogeogr. 41:1379–1389.

Delava, E., F. Fleury, and P. Gibert. 2016. Effects of daily fluctuating temperatures on the Drosophila–*Leptopilina boulardi* parasitoid association. J. Therm. Biol. 60:95–102. Elsevier.

Delpuech, A. J., B. Moreteau, J. Chiche, E. Pla, and J. R. David. 1995. Phenotypic Plasticity and Reaction Norms in Temperate and Tropical Populations of *Drosophila melanogaster* : Ovarian Size and Developmental Temperature Published by : Society for the Study of Evolution Stable URL : http://www.jstor.org/stable/2410320 . PHE. Evolution (N. Y). 49:670–675.

Drown, D. M., E. P. Levri, and M. F. Dybdahl. 2011. Invasive genotypes are opportunistic specialists not general purpose genotypes. Evol. Appl. 4:132–143.

Engel, K., R. Tollrian, and J. M. Jeschke. 2011. Integrating biological invasions, climate change, and phenotypic plasticity. Commun. Integr. Biol. 4:247–250.

Estoup, A., V. Ravigné, R. Hufbauer, R. Vitalis, M. Gautier, and B. Facon. 2016. Is There A Genetic Paradox of Biological Invasion? Annu. Rev. Ecol. Evol. Syst. 47:51–72.

Fleury, F., P. Gibert, N. Ris, and R. Allemand. 2009. Ecology and life history evolution of frugivorous Drosophila parasitoids. Adv. Parasitol. 70:3–44.

Foray, V., P.-F. Pelisson, M.-C. Bel-Venner, E. Desouhant, S. Venner, F. Menu, D. Giron, and B. Rey. 2012. A handbook for uncovering the complete energetic budget in insects: the van Handel's method (1985) revisited. Physiol. Entomol. 37:295–302.

Frei, E. R., J. Ghazoul, and A. R. Pluess. 2014. Plastic Responses to Elevated Temperature in Low and High Elevation Populations of Three Grassland Species. PLoS One 9:e98677.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new

492 environments. Funct. Ecol. 21:394–407.

493 Gibert, P., M. Hill, M. Pascual, C. Plantamp, J. S. Terblanche, A. Yassin, and C. M. Sgrò.
494 2016. Drosophila as models to understand the adaptive process during invasion. Biol.
495 Invasions 18:1089–1103.

496 Godfray, H. C. J. 1994. Parasitoids, behavioral and evolutionary ecology. Princeton
497 University Press, Princeton.

498 Gotthard, K., S. Nylin, and S. Nylin. 1995. Adaptive Plasticity and Plasticity as an
499 Adaptation: A Selective Review of Plasticity in Animal Morphology and Life History.
500 Oikos 74:3.

501 Hamilton, J. A., M. Okada, T. Korves, and J. Schmitt. 2015. The role of climate adaptation in
502 colonization success in *Arabidopsis thaliana*. Mol. Ecol. 24:2253–2263.

503 Hertlein, M. 1986. Seasonal development of Leptopilina boulardi (Hymenoptera: Eucolidae)
504 and its hosts. *Drosophila melanogaster* and *Drosophila simulans* (Diptera:
505 Drosophilidae) in California. Environ. Entomol. 15:859–866.

506 Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. Nature
507 470:479–485.

508 Hulme, P. E. 2008. Phenotypic Plasticity and Plant Invasions: Is It All Jack? Funct. Ecol.
509 22:3–7.

510 Johnson, J. B., and K. S. Omland. 2004. Model selection in ecology and evolution. Trends
511 Ecol. Evol. 19:101–8.

512 Lelièvre, F., S. Sala, F. Ruget, and V. F. 2011. Evolution climatique du sud de la France
513 1950-2009. CLIMFOUREL PSDR-3 Proj. Reg. L-R, M-P, R-A. Les Focus PSDR3.
514 Available https//www6.inra.fr/psdr-midi-pyrenees/Qu-est-ce-que-PSDR/Archives-
515 PSDR-3/Productions-operationnelles/Focus/Focus-PSDR3-Climfourel-Evolution-du-
516 climat-du-Sud-de-la-France-1.

517 Lind, M. I., and F. Johansson. 2011. Testing the role of phenotypic plasticity for local
518 adaptation: growth and development in time-constrained *Rana temporaria* populations.
519 J. Evol. Biol. 24:2696–2704.
520 Moiroux, J., E. Delava, F. Fleury, and J. van Baaren. 2013. Local adaptation of a Drosophila
521 parasitoid: habitat-specific differences in thermal reaction norms. J. Evol. Biol. 26:1108–
522 16.
523 Morin, J. P., B. Moreteau, G. Pétavy, and J. R. David. 1999. Divergence of reaction norms of
524 size characters between tropical and temperate populations of *Drosophila melanogaster*
525 and D. simulans. J. Evol. Biol. 12:329–339.
526 Nicol, C. M. Y., M. Mackauer, B. Sciences, and C. Va. 1999. The scaling of body size and
527 mass in a host-parasitoid association : influence of host species and stage. 83–92.
528 Nicotra, A. B., O. K. Atkin, S. P. Bonser, A. M. Davidson, E. J. Finnegan, U. Mathesius, P.
529 Poot, M. D. Purugganan, C. L. Richards, F. Valladares, and M. van Kleunen. 2010. Plant
530 phenotypic plasticity in a changing climate. Trends Plant Sci. 15:684–692. Elsevier Ltd.
531 Nordlander, G. 1980. Revision of the genus *Leptopilina* Förster; 1869, with notes on the
532 status of some other genera (Hymenoptera, Cynipodea, Eucoilidae). Entomol. Scand.
533 11:428–453.
534 Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts
535 across natural systems. Nature 421:37–42.
536 Patot, S., D. Lepetit, D. Charif, J. Varaldi, and F. Fleury. 2009. Molecular detection,
537 penetrance, and transmission of an inherited virus responsible for behavioral
538 manipulation of an insect parasitoid. Appl. Environ. Microbiol. 75:703–10.
539 Patot, S., J. Martinez, R. Allemand, S. Gandon, J. Varaldi, and F. Fleury. 2010. Prevalence of
540 a virus inducing behavioural manipulation near species range border. Mol. Ecol. 2995–
541 3007.

542 Pelosse, P., C. Bernstein, and E. Desouhant. 2007. Differential energy allocation as an
543 adaptation to different habitats in the parasitic wasp *Venturia canescens*. Evol. Ecol.
544 669–685.
545 Pereira, R. J., M. C. Sasaki, and R. S. Burton. 2018. Adaptation to a latitudinal thermal
546 gradient within a widespread copepod species: the contributions of genetic divergence
547 and phenotypic plasticity. Proceedings. Biol. Sci. 284:20170236.
548 Prentis, P. J., J. R. U. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008.
549 Adaptive evolution in invasive species. Trends Plant Sci. 13:288–94.
550 Richards, C. L., O. Bossdorf, N. Z. Muth, J. Gurevitch, and M. Pigliucci. 2006. Jack of all
551 trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol.
552 Lett. 9:981–93.
553 Ris, N., R. Allemand, P. Fouillet, and F. Fleury. 2004. The joint effect of temperature and
554 host species induce complex genotype-by-environment interactions in the larval
555 parasitoid of Drosophila, *Leptopilina heterotoma* (Hymenoptera: Figitidae). Oikos
556 106:451–456.
557 Rivero, A., and J. Casas. 1999. Incorporating physiology into parasitoid behavioral ecology :
558 the allocation of nutritional resources. Res. Popul. Ecol. 41:39–45.
559 Root, T. L., J. T. Price, K. R. Hall, and S. H. Schneider. 2003. Fingerprints of global warming
560 on wild animals and plants. Nature 57–60.
561 Scoville, A. G., and M. E. Pfrender. 2010. Phenotypic plasticity facilitates recurrent rapid
562 adaptation to introduced predators. Proc. Natl. Acad. Sci. 107:4260–4263.
563 Sgrò, C. M., J. S. Terblanche, and A. A. Hoffmann. 2016. What Can Plasticity Contribute to
564 Insect Responses to Climate Change? Annu. Rev. Entomol. 61:433–452.
565 Urbanski, J., M. Mogi, D. O'Donnell, M. DeCotiis, T. Toma, and P. Armbruster. 2012. Rapid
566 Adaptive Evolution of Photoperiodic Response during Invasion and Range Expansion

567 across a Climatic Gradient. Am. Nat. 179:490–500.

568 Varaldi, J., M. Boulétreau, and F. Fleury. 2005. Cost induced by viral particles manipulating

569 superparasitism behaviour in the parasitoid *Leptopilina boulardi*. Parasitology 131:161–

570 168.

571 Varaldi, J., P. Fouillet, M. Ravallec, M. Lopez-Ferber, M. Boulétreau, and F. Fleury. 2003.

572 Infectious behavior in a parasitoid. Science (80-.). 302:1930.

573 Visser, B., and J. Ellers. 2008. Lack of lipogenesis in parasitoids: A review of physiological

574 mechanisms and evolutionary implications. J. Insect Physiol. 54:1315–1322.

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Figures

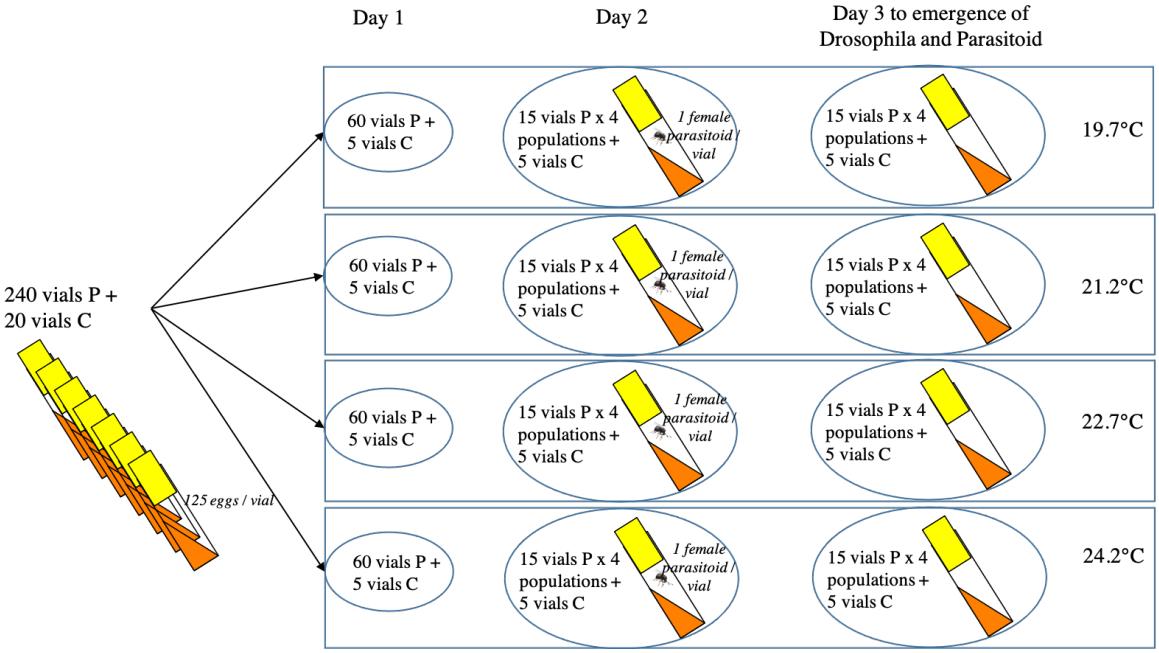


Figure 1. Schematic representation of the experimental protocol used: 125 D. melanogaster are deposited in each tube in which a female *L. boulardi* is placed for 24H. 15 replicates are made for each population and each thermal regime. For each condition, 5 control tubes without parasitoids are used to evaluate the quality of Drosophila development.

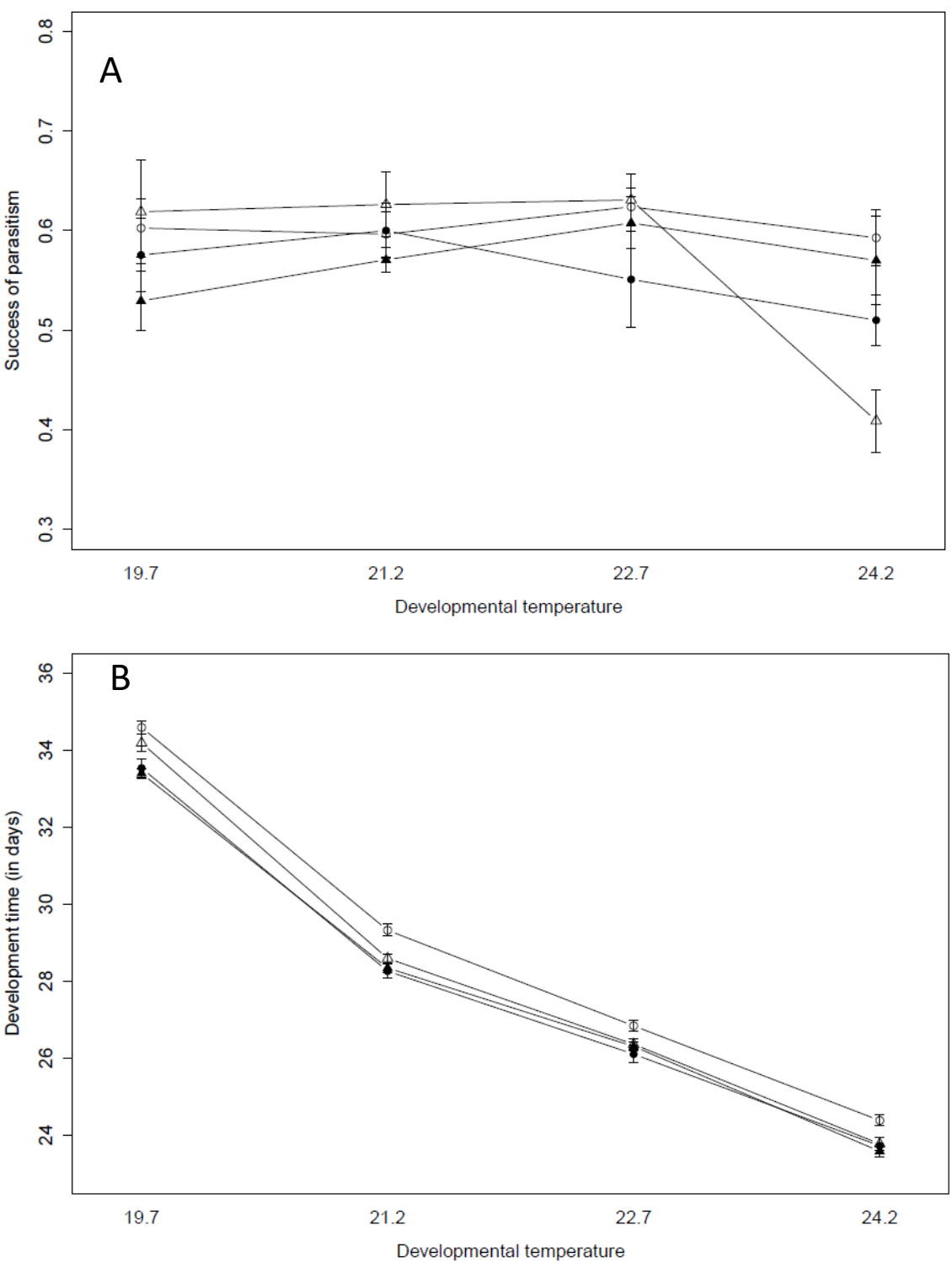
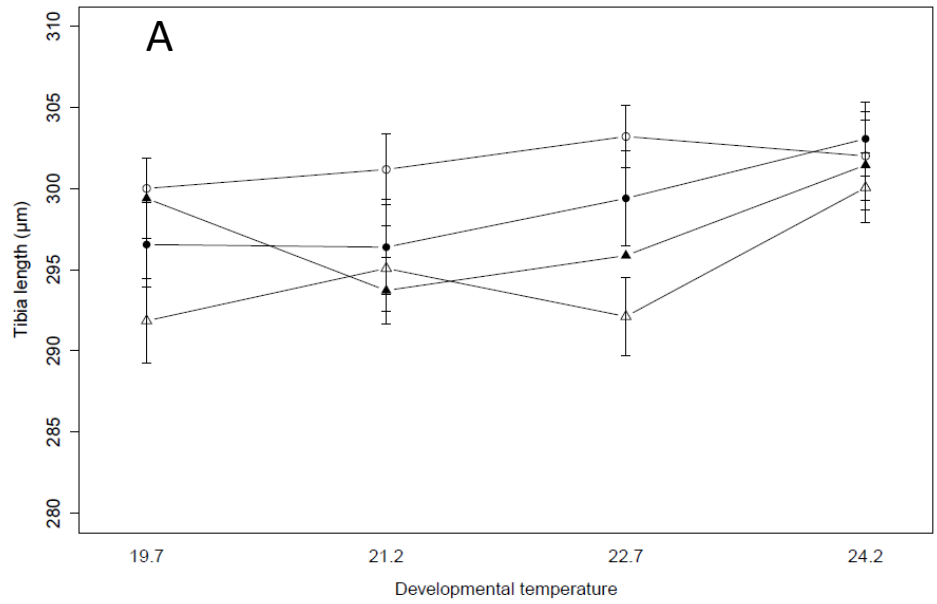


Figure 2. Reaction norms of A) success of parasitism and B) development time across the four fluctuating developmental thermal regime. Each point represents the mean value per population (± SE). Open symbols represent the two core populations (triangle: S2 and circle: S1) and symbols in black represent the two marginal populations (triangle: N2 and circle: N1).



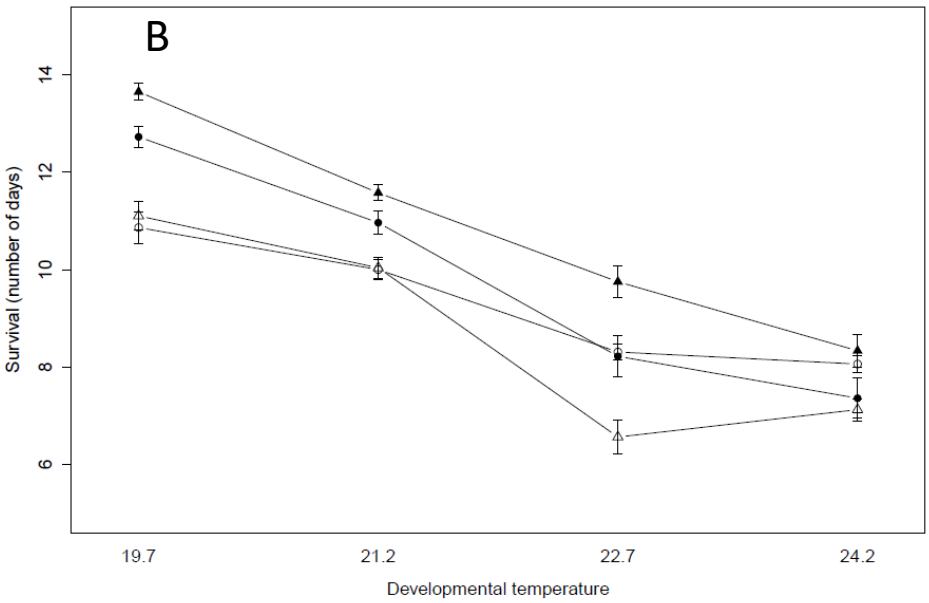


Figure 3 Tibia length A) and starvation resistance B) (mean±SE) reaction norms across four developmental thermal regimes. Open symbols represent the two core populations (triangle: S2 and circle: S1) and symbols in black represent the two marginal populations (triangle: N2 and circle: N1).

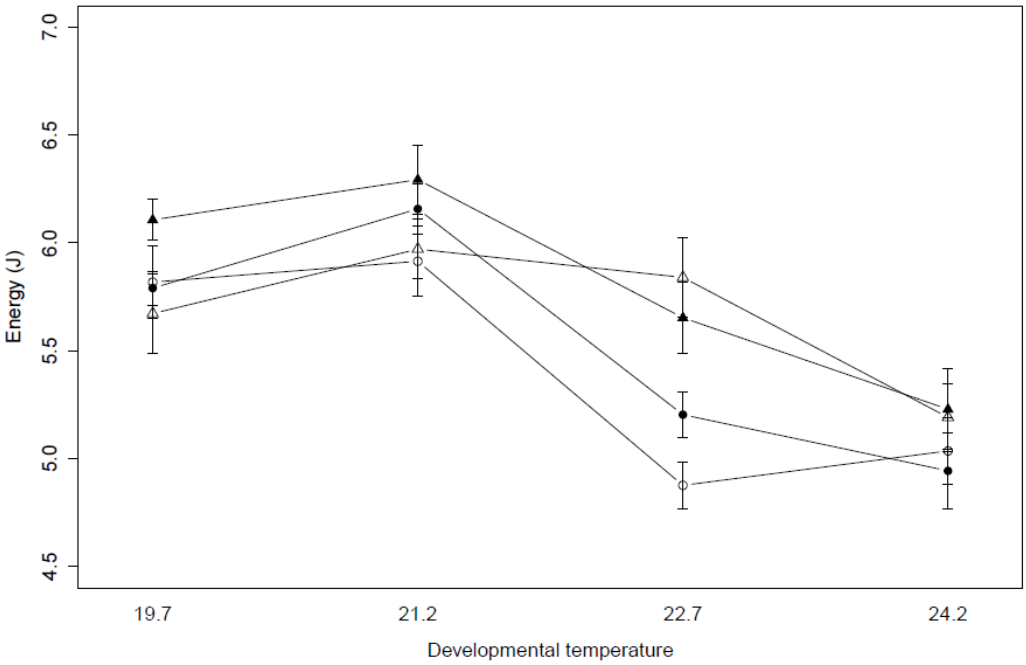


Figure 4. Energy content (mean±SE) reaction norms across four developmental thermal regimes. Open symbols represent the two core populations (triangle: S2 and circle: S1) and symbols in black represent the two marginal populations (triangle: N2 and circle: N1).

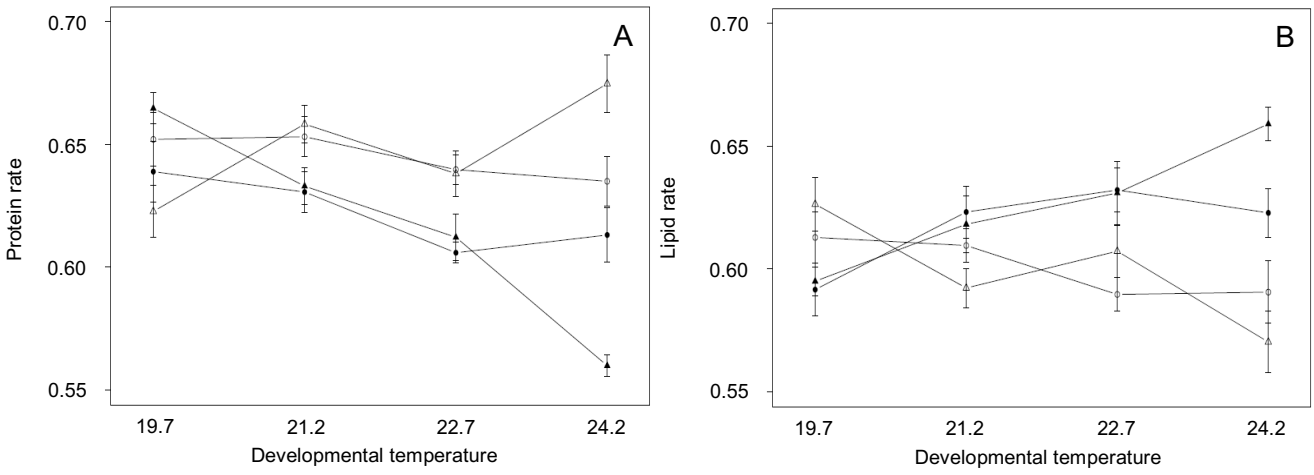


Figure 5 A) protein rate and B) lipid rate (mean±SE) reaction norms across four developmental temperatures. Open symbols represent the two core populations (triangle: S2 and circle: S1) and symbols in black represent the two marginal populations (triangle: N2 and circle: N1).