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1 **Are life-history traits equally affected by global warming? A case study**

2 **combining a multi-trait approach with fine-grain climate modeling**

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12 **Abstract**

13 Predicting species responses to climate change requires tracking the variation in individual
14 performance following exposure to warming conditions. One ecologically relevant approach
15 consists of examining the thermal responses of a large number of traits, both related with
16 population dynamics and trophic interactions (i.e. a multi-trait approach). Based on *in situ*
17 climatic data and projections from climate models, we here designed two daily fluctuating
18 thermal regimes realistically reflecting current and future conditions in Eastern France. These
19 models detected an increase in mean temperature and in the range of daily thermal
20 fluctuations as two local facets of global warming likely to occur in our study area by the end
21 of this century. We then examined the responses of several fitness-related traits in caterpillars
22 of the moth *Lobesia botrana* – including development, pupal mass, survival rates, energetic
23 reserves, behavioral and immune traits expressed against parasitoids – to this experimental
24 imitation of global warming. Increasing temperatures positively affected development
25 (leading to a 31% reduction in the time needed to complete larval stage), survival rates
26 (+19%), and movement speed as a surrogate for larval escape ability to natural enemies
27 (+60%). Conversely, warming elicited detrimental effects on lipid reserves (-26%) and
28 immunity (total phenoloxidase activity: -34%). These findings confirm that traits should differ
29 in their sensitivity to global warming, underlying complex consequences for population
30 dynamics and trophic interactions. Our study strengthens the importance of combining a
31 multi-trait approach with the use of realistic fluctuating regimes to forecast the consequences
32 of global warming for individuals, species and species assemblages.

33 **Keywords**

34 Climate models, defensive behaviors, energetic reserves, global warming, immunity, multi-
35 trait approach.

36 **1. Introduction**

37 Temperature is undeniably one of the most important factors determining individual
38 performance, species evolution and distribution (Angilletta, 2009; Clarke, 2003). In
39 ectothermic animals like insects, whose body temperature and performance are closely related
40 with environmental temperature, the link between a surrogate for individual performance (e.g.
41 development and growth rates, reproductive output) and environmental temperature is
42 typically modeled by an asymmetric and unimodal curve called a thermal performance curve
43 (Martin and Huey, 2008; Sinclair et al., 2016). As temperature increases, individual
44 performance gently rises to a single maximum value associated with a thermal optimum, and
45 thereafter performance declines rapidly.

46 Thermal performance curves may help to predict how organisms will cope with
47 changes occurring in their thermal environment, such as those related with anthropogenic
48 climate disturbance (Deutsch et al., 2008; Estay et al., 2014; Sinclair et al., 2016). Indeed, one
49 commonly used way to assess the biological consequences of global warming consists of
50 experimentally tracking the variation in organism's performance following exposure to
51 warming conditions designed to simulate future temperatures (for examples, see Bauerfeind
52 and Fischer, 2014; Fischer et al., 2014; Klockmann et al., 2016). In this regard, it is now
53 widely accepted that comparing fluctuating thermal regimes (instead of constant
54 temperatures) significantly improves the ecological realism of global warming experiments,
55 considering the daily variations of temperatures experienced by organisms in their natural
56 settings (Bozinovic et al., 2016, 2011; Colinet et al., 2015; Paaijmans et al., 2013; Zeh et al.,

2015). The existing literature suggests complex patterns of biological responses to warmer conditions because of the combined effects of mean temperature and thermal fluctuations on individual performance (Colinet et al., 2015; Estay et al., 2014; Kingsolver et al., 2015). Part of this variability in thermal responses might be explained by Jensen's inequality, which applies to non-linear functions such as those inherent to thermal performance curves (Martin and Huey, 2008). Jensen's inequality posits that thermal fluctuations around a mean temperature below the thermal optimum (i.e. in the concave part of the thermal performance curve) will enhance performance, while the reverse trend should be observed once the mean temperature of the thermal regime exceeds the thermal optimum, in the convex part of the thermal performance curve (Bozinovic et al., 2011; Foray et al., 2014; Kingsolver et al., 2015; Paaijmans et al., 2013; Terblanche et al., 2010). In a context of fluctuating thermal environments, an increase in mean temperature attributable to global warming could be expected to depress organism performance through transient or permanent exposure to stressful thermal conditions during the warmest hours of the cycle (Colinet et al., 2015; Paaijmans et al., 2013; Vasseur et al., 2014). This is especially true if the increase in mean temperature is accompanied with a widening of thermal fluctuations (Paaijmans et al., 2013; Terblanche et al., 2010; Vasseur et al., 2014). Indeed, high levels of temperature variance have been shown to decrease an organism's ability to tolerate a thermal stress by limiting the expression of plastic responses and reducing the possibility for appropriate acclimation process to take place (Terblanche et al., 2010).

Responses to warming conditions might, however, greatly vary depending on the biological traits measured to infer individual performance, thereby highlighting the need for a multi-trait approach – involving measurements on a large number of traits – in global warming experiments (Fischer et al., 2011; Karl et al., 2011; Laughton et al., 2017). Indeed, fitness-related traits in a given species might differ regarding their thermal optima and

82 associated thermal performance curves (Laughton et al., 2017; Seehausen et al., 2017).
83 Experimental warming might thus yield different effects on the expression of these traits
84 depending on whether thermal conditions applied encompass supraoptimal temperatures for
85 the expression of the traits considered. For instance, in the Indian meal moth *Plodia*
86 *interpunctella* (Lepidoptera: Pyralidae), a rise in mean constant temperature from 20°C to
87 30°C led to an almost twofold decrease in developmental time from egg to adult, while
88 reducing adult lifespan after mating by 32% and increasing the count of immune cells
89 (hemocytes) in larvae hemolymph by more than 50% (Laughton et al., 2017). Furthermore,
90 biological traits are linked to each other by energetic trade-offs and thermal conditions are
91 known to influence resource allocation towards different functions. Convincing examples
92 entail trade-offs between immunity and reproduction (Adamo and Lovett, 2011) as well as
93 between immunity and heat tolerance (Fischer et al., 2011; Karl et al., 2011). Experimental
94 studies employing a multi-trait approach usually incorporate both traits linked with population
95 dynamics (e.g. development and growth rates, survival rates, egg production) and
96 interspecific relationships (e.g. defensive traits such as defensive behaviors and immunity)
97 (Iltis et al., 2018; Karl et al., 2011; Laughton et al., 2017). Indeed, the former help to predict
98 the future population dynamics of the focal species, but species will not respond in isolation
99 to climate disturbance as they are tightly connected by ecological linkages, more specifically
100 trophic interactions (Jeffs and Lewis, 2013; Van der Putten et al., 2010). Integrating defensive
101 traits involved in interspecific relationships – such as host-parasitoid associations – is thus
102 crucial to provide significant insight into how a given species might respond to global
103 warming with respect to its associated ecological context (Iltis et al., 2018). In spite of a fairly
104 robust literature about the effects of thermal fluctuations on performance in a climate change
105 perspective, still few studies have to date associated the ecological realism provided by the
106 use of realistic fluctuating regimes with the multi-trait approach, measuring different traits

107 related with both population dynamics and species interaction (for examples, see Fischer et
108 al., 2014; Klockmann et al., 2016). Consequently, little is known about the effects of realistic
109 daily thermal cycles on the expression of a broad series of traits within the framework of
110 global warming. Filling this knowledge gap could improve our mechanistic understanding of
111 the responses of traits, individuals and populations to facets of climate change.

112 In this context, we here explored the consequences of global warming simulated at a
113 local scale for several aspects of an insect's performance. To realistically simulate global
114 warming under controlled conditions, we built two daily fluctuating regimes: one current
115 regime according to local climate observations and one future regime according to the fine-
116 grain predictions provided by climate models in our focal area (Eastern France). We focused
117 on caterpillars of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae),
118 one of the most severe pests for wine-making activity in Palearctic region (Thiéry et al.,
119 2018). We adopted a global view of the insect's performance encompassing a large number of
120 fitness-related traits, such as development time, pupal mass, survival rates, and the storage
121 patterns of four energetic compounds. In a wider ecological context, we also focused on the
122 abilities of the caterpillars to survive in agroecosystems when faced with natural enemies,
123 especially parasitoids, by examining larval defensive strategies that involve behavioral
124 defenses (aimed at avoiding parasitoid oviposition) and immune defenses (aimed at killing
125 parasitoid eggs) (Iltis et al., 2018; Vogelweith et al., 2014).

126 We hypothesized that biological traits included in this study should respond in
127 different ways to our simulation of future climates that might be experienced in the same
128 location. Given its tight dependence on environmental temperature as a pure physiological
129 rate (Rebaudo and Rabhi, 2018), we expected larval development to be greatly reduced by
130 warming. The thermal response of larval development should in turn determine the time
131 allowed for feeding in this capital breeding species (i.e. only caterpillars feed), thereby

132 negatively impacting pupal mass and energetic reserves. We expected defensive behaviors to
133 display more complex patterns of responses to warming, as the thermal modulation of insect
134 behavior is not purely metabolic, that is, does not only arise from short-term variation in
135 metabolic rates or long-term variation in individual physiological state (e.g. amounts and
136 allocation of energetic reserves) ('kinetic effects', Abram et al., 2017). It also involves
137 intentional behavioral adjustments in response to the perception and the integration of thermal
138 information received by thermosensory organs ('integrated effects', Abram et al., 2017). All of
139 these possible effects were included in our experimental design which sought to investigate
140 the overall impacts of warming on the expression of defensive behaviors to infer the future
141 ability of caterpillars to fend off parasitoids. Finally, we postulated that immunity should be
142 negatively affected by high temperatures because immune function in *L. botrana* caterpillars
143 is known to peak at a relatively low thermal optimum (22°C or less) (Iltis et al., 2018).

144 **2. Material and methods**

145 *2.1 Thermal regimes*

146 We compared the performance of *L. botrana* caterpillars experiencing two daily fluctuating
147 thermal regimes representative of current and future conditions occurring in Burgundy,
148 Eastern France (Longvic-Dijon weather station, 47,27°N; 5,09°E; altitude = 219 m)
149 (www.meteofrance.com). We focused on the summer period extending from 15th July to 15th
150 August because this one-month period is particularly favorable for *L. botrana*, as indicated by
151 one peak of activity (adult moths emergence) over this date range (Barnay et al., 2001;
152 Martín-Vertedor et al., 2010). A curve of current thermal conditions was designed according
153 to a 20-year recording of *in situ* hourly temperatures (from 1995 to 2014). Based on this
154 curve, we built a mean daily cycle divided into six segments, each lasting 4 h and calculated

155 as the mean segment over the 30 days (15th July - 15th August) and the 20-year period studied
156 (Table 1). Hereafter, this thermal regime will be referred to as the current regime.

157 Alongside the current regime, we built the future regime simulating the local
158 conditions that might be found in Burgundy by the end of this century (2081-2100), during
159 the summer period extending from 15th July to 15th August. We selected the most pessimistic
160 of the Representative Concentration Pathways scenarios, called RCP 8.5, which predicts the
161 highest positive radiative forcing (i.e. a high absorbance of solar and infrared radiation)
162 associated with massive and growing emissions of greenhouse gas (www.drias-climat.fr).
163 This scenario was combined with six simulations of high-resolution mesh (12.5 km) applied
164 to the closest point to Dijon (Burgundy). Each simulation was obtained by downscaling a
165 General Circulation Model (GCM) with a Regional Climate Model (RCM) to increase the
166 spatial accuracy of climate predictions. Both GCMs and RCMs are climate tools that enable
167 to follow the evolution of a climate system over time, at different spatial scales: global scale
168 for GCMs, regional or local scale for RCMs nested within GCMs (for more details about the
169 use of climate models in ecology, see Beaumont et al., 2008; Ziter et al., 2012). The six
170 combinations of GCM/RCM used were: CERFACS-CNRM/RCA4, ICHEC-EC-
171 EARTH/RCA4, MPI-ESM-LR/CCLM4, MPI-ESM-LR/REMO019, MPI-ESM-LR/RCA4,
172 MetEir-ECEARTH/RACMO22E. These combinations have been recently used to generate
173 simulations of climate change at small spatial scales across Europe (Alfieri et al., 2015;
174 Dosio, 2016; Kotlarski et al., 2014). We used GCMs originating from the Coupled Model
175 Intercomparison Project Phase 5 (CMIP5), a recent multi-model ensemble widely used to
176 produce climate projections in the context of anthropogenic climate change (Taylor et al.,
177 2012). All the RCMs used to generate downscaled simulations came from the Coordinated
178 Regional Climate Downscaling Experiment across Europe (EURO-CORDEX) and were
179 adapted to downscale the outputs of the GCMs used (Dosio, 2016; Jacob et al., 2014). All

180 data are available on the Drias portal (www.drias-climat.fr). For each simulation, daily
181 maximum and minimum air temperatures were extracted. We then generated six future mean
182 daily cycles (one for each simulation) based on simulated maximum and minimum
183 temperatures and the present daily cycle. The future regime, as the current regime, was
184 composed of six segments of 4 h, each calculated as the mean segment over the six
185 simulations performed (Table 1). Comparison between current and future regimes reveals that
186 global warming should increase both the mean temperature (+ 5.3°C) and the daily thermal
187 range (+ 0.6°C) in our focal area (Table 1).

188 **Table 1** Temperature conditions (°C) for the two daily fluctuating regimes used in this study:
189 the current regime and the future regime, each divided into 6 segments of 4 h. Daily thermal
190 range is defined as the difference between maximum and minimum daily temperatures.

	Current regime	Future regime
0h – 4h	16.4	21.4
4h – 8h	17.8	22.9
8h – 12h	22.3	27.8
12h – 16h	24.9	30.5
16h – 20h	22.9	28.3
20h – 0h	19.0	24.2
Mean temperature	20.5	25.8
Daily thermal range	8.5	9.1

191

192 *2.2 Insect stock and larvae collection*

193 We used larvae originating from an inbred and diapause free strain of *L. botrana* maintained
194 at the French National Institute for Agricultural Research (INRA, Villenave d'Ornon, France)
195 for several years under controlled conditions. This stock consists of a large number of caged
196 adults (several thousand per week), to which wild individuals were regularly added to
197 preserve genetic diversity. Hence, larvae coming from this artificial stock display similar
198 patterns of defensive behaviors and basal immunity compared with wild individuals collected
199 in French vineyards (Vogelweith et al., 2014). Moths were reared under standard conditions
200 ($20 \pm 0.5^\circ\text{C}$, $60 \pm 5\%$ relative humidity, photoperiod of L17: D6 and 1 h of dusk). Luminosity
201 was 600 lx during photophase and 100 lx at dusk. Two bands of waxed paper were provided
202 as a support for oviposition. Every 2 days, these papers were renewed and transferred to a
203 plastic box covered by a moist piece of paper towel to avoid egg desiccation until hatching.

204 A total of 2246 freshly-hatched larvae (aged < 24 h) were collected throughout the
205 experiments with a fine brush and individually reared in Eppendorf tubes filled with 1.5 ml of
206 artificial medium (composition for 1000 ml: 1000 ml water, 15 g agar, 86.6 g maize flour,
207 41.3 g wheat germ, 45.5 g beer yeast, 6 g ascorbic acid, 3.4 g mineral salt, 0.32 g Scala[®], 2.7
208 g benzoic acid, 2.8 g Nipagin[®] and 5 ml 95% ethanol) (Thiéry and Moreau, 2005). Neonate
209 caterpillars were isolated in tubes so that no larval competition could occur and introduce an
210 experimental bias (Thiéry et al., 2014). Tube caps were pierced and covered by a piece of fine
211 mesh fabric to allow air circulation while preventing larvae from escaping. Individuals were
212 randomly assigned to two incubators (ST 2/2 BASIC, Pol-Eko Aparatura), each simulating
213 one of the two thermal regimes described above (temperature $\pm 0.1^\circ\text{C}$, $50 \pm 10\%$ relative
214 humidity, L18: D6, 650 lx). In order to avoid a potential incubator effect, larvae and their
215 associated thermal regime were switched between climate chambers two times a week, as
216 well as the location of individuals inside the new incubator. Temperatures within the climate

217 chambers were tracked weekly with an independent data logger (Hobo, Onset Computer
218 Corporation), and did not deviate from the thermal regimes programmed during the whole
219 course of the experiments. Larval development was surveyed when caterpillars were swapped
220 between incubators (i.e. two times a week) to minimize disturbance. Once they reached their
221 fifth – and final – instar stage, larvae were randomly assigned to one of three experimental
222 groups all along the experiments, because the different measurements of biological traits
223 involved in this study cannot be taken on a single individual. We focused on the fifth instar
224 because the energetic resources accumulated during the whole larval stage can be quantified
225 at this late larval instar that directly precedes pupation. In addition, measurements of
226 behavioral and immune defenses against parasitoids are classically performed at the end of
227 larval development in this species (Iltis et al., 2018; Vogelweith et al., 2014). In Group 1, the
228 fifth instar larvae were left until pupation in order to measure development time, pupal mass,
229 survival from first instar larva to adult stage, and sex ratio. In Group 2, the fifth instar larvae
230 were frozen and stored to quantify later their energy budgets. In Group 3, the behavioral and
231 immune traits involved in defense of fifth instar caterpillars against larval parasitoids were
232 measured.

233 *2.3 Group 1: development time, pupal mass, survival and sex ratio*

234 A total of 2043 freshly hatched larvae were initially assigned to Group 1 in order to develop
235 until pupation ($n = 981$ individuals for current regime, $n = 1062$ individuals for future
236 regime). We recorded the time (in days) elapsed between hatching and pupation (i.e. the
237 development time of the larval stage), which corresponds to the time during which caterpillars
238 are able to feed. The pupae were collected carefully with fine forceps and weighed (± 0.1 mg)
239 with a balance (Pioneer PA214C, OHAUS). Development time and pupal mass were only
240 recorded for individuals that reached the adult stage to discriminate the results between males
241 and females, which were sexed upon emergence. We also calculated survival (number of

242 emerged adults relative to the number of freshly hatched larvae initially assigned to each
243 thermal regime), and sex ratio (expressed as the proportion of males in the adult population).

244 *2.4 Group 2: energetic assessments*

245 These measurements initially involved 59 fifth instar larvae ($n = 29$ for current regime, $n = 30$
246 for future regime). Caterpillars were cleaned with a fine brush to remove any residual of
247 artificial medium from the surface of their body, transferred to Eppendorf tubes, frozen in
248 liquid nitrogen and kept at -80°C for latter quantification of their energy budgets. They were
249 subsequently weighed to obtain fresh body mass (± 0.01 mg) with a microbalance (Quintix
250 35-15, Sartorius), before being crushed with stainless steel beads (tubes shaken for 90 s at 25
251 Hz) in 180 μl of aqueous lysis buffer solution (composition: 100 mM KH_2PO_4 , 1 mM
252 dithiothreitol DTT, 1 mM ethylenediaminetetraacetic EDTA, pH = 7.4). Biochemical assays
253 were run on four metabolites potentially involved in energy production: proteins, lipids,
254 soluble carbohydrates and glycogen. The total amount of soluble proteins available was
255 estimated using a DC Protein Assay kit (Bio-Rad). We used the spectrophotometry method
256 described by Foray et al. (2012) for the quantification of lipids, soluble carbohydrates and
257 glycogen.

258 Crushed samples were placed on ice for 30 min to ensure proteins solubilization,
259 centrifuged (4000 g, 5 min, 4°C) and 5 μl of supernatant from each sample were pipetted to
260 microplate wells together with 35 μl of lysis buffer to obtain diluted samples. Particular
261 attention was paid to avoid collecting lipids (which often form a thin surface layer on the top
262 of the sample) when pipetting the 5 μl of supernatant for protein assays, because lipids might
263 disturb the absorbance readings in the Bradford approach used to quantify proteins (Foray et
264 al., 2012). Then, 5 μl of these diluted samples were transferred to new microplate wells and
265 supplemented with 225 μl of Bradford micro-assay reagent (DC Protein Assay Kit, Bio-Rad).

266 Reaction was allowed to proceed for 15 min at room temperature, after which absorbance
267 readings were taken at 750 nm. Protein concentration in the samples was estimated with a
268 standard curve based on a dilution-series of bovine gamma globulin standard (Pierce, Thermo
269 Fisher Scientific) dissolved in lysis buffer. This dilution-series was constituted by the
270 following protein concentration values: 0, 0.062, 0.125, 0.25, 0.5, 1 and 2 mg.ml⁻¹. Finally, 5
271 µl of lysis buffer, 20 µl of 20 % sodium sulphate solution and 1.5 ml of chloroform-methanol
272 mixture (1:2 v/v) were added to each crushed sample to solubilize total lipids and water-
273 soluble carbohydrates. These samples were then conserved at room temperature for 24h
274 before biochemical assays of the amounts of lipids, soluble carbohydrates and glycogen.

275 At the end of this 24h duration, homogenates were centrifuged at room temperature
276 (4800 g, 6 min). Centrifugation enabled to isolate lipids and soluble carbohydrates
277 (supernatant) from glycogen (pellet). Determination of lipid body amount was performed with
278 a colorimetric method involving vanillin reagent (Alfa Aesar, Thermo Fischer Scientific). For
279 each centrifuged sample, 75 µl of supernatant were transferred to an Eppendorf tube and
280 heated at 90°C until complete solvent evaporation. Then, each tube was supplemented with 40
281 µl of 95-98% sulphuric acid and incubated at 90°C for 10 min. For lipids revelation, 960 µl of
282 vanillin solution (composition: 100 ml ultrapure water, 400 ml 85% orthophosphoric acid,
283 600 mg vanillin) were then deposited in each tube. After vigorous vortexing, 200 µl of each
284 tube were pipetted to microplate wells for absorbance measurements, which were taken at 525
285 nm. The dilution-series used to assess lipid concentration in the samples involved glyceryl
286 trioleate as a standard (Sigma-Aldrich) dissolved in chloroform, and was comprised by the
287 following concentration values: 0, 2.5, 5, 10, 20, 40 and 80 µg.ml⁻¹. The body amounts of
288 both soluble carbohydrates and glycogen were determined with a colorimetric method based
289 on anthrone reagent (Alfa Aesar, Thermo Fisher Scientific). For soluble carbohydrates, 750 µl
290 of supernatant from each centrifuged sample were collected and deposited in an Eppendorf

291 tube, before being allowed to fully evaporate at 95°C. For glycogen, the remaining
292 supernatant in the centrifuged samples was removed and the pellet was left at room
293 temperature during 30 min for complete solvent evaporation. Then, 2 ml of anthrone solution
294 (composition: 150 ml ultrapure water, 380 ml 95-98% sulfuric acid, 750 mg anthrone) were
295 added to each tube (containing either soluble carbohydrates or glycogen), and all these tubes
296 were heated at 95°C for 17 min. Tubes were subsequently vortexed and 200 µl of each were
297 withdrawn and deposited in microplate wells before readings of optical density, which were
298 performed at 625 nm. The concentration of these two energetic compounds in the samples
299 was estimated through comparison with a dilution-series constituted by the following
300 concentration values of glucose (Sigma-Aldrich): 0, 6.25, 12.5, 25, 50, 100, 200 and 400
301 µg.ml⁻¹.

302 For proteins and lipids, two replicates were performed and individuals for which the
303 coefficient of variation exceeded 30% were excluded from further analysis. Repeatability was
304 relatively high, as indicated by the low percentages of individuals removed from the protein
305 (8.5%) and the lipid datasets (5.1%). At the end of this selection procedure, we retained 54
306 larvae from the protein dataset ($n = 27$ for current regime, $n = 27$ for future regime) and 56
307 larvae from the lipid dataset ($n = 29$ for current regime, $n = 27$ for future regime).

308 *2.5 Group 3: behavioral and immune defenses*

309 A total of 144 fifth instar larvae were assigned to measurements of defensive traits ($n = 73$
310 individuals for current regime, $n = 71$ individuals for future regime). Three successive
311 behaviors were quantified, always in the same order ('flee', 'twisting' and 'dropping'), in
312 accordance with the sequence involved in defense against larval parasitoids in natural
313 conditions (Vogelweith et al., 2014). Additionally, these behavioral tests were performed at
314 an ambient temperature strictly identical to the mean temperature of the regime considered:

315 20.5°C if the tested individual was reared in current regime, or 25.8°C if it was reared in
316 future regime (Table 1). Temperature in the testing rooms was controlled with Hobo data
317 loggers. After the completion of each behavioral test, 20 min of rest were given to each larva
318 in a clean Eppendorf tube drilled to allow air circulation before the next test started.

319 The first behavior studied, called ‘flee’, refers to larvae escape ability by moving away
320 from a generic threat without leaving the bunch they feed on. Here, we estimated the
321 locomotor activity (movement speed) of caterpillars as a proxy of ‘flee’ behavior, to infer
322 their ability to evade parasitoids in natural conditions. For this purpose, caterpillars were
323 installed on a plastic squared sheet (84 x 116 cm, squares size: 1 x 1 cm) and acclimated for
324 30 s under the lid of a Petri dish. The lid was then removed and each larva was observed to
325 count the total number of lines crossed during 60 s (the minimum duration required by a larva
326 to exit the sheet). As this behavior occurs before physical contact between the caterpillar and
327 the parasitoid, no simulation of a parasitoid attack was needed to cause the larvae to flee.
328 Locomotor activity was naturally induced by the stress associated with the manipulation of
329 caterpillars with a fine paintbrush during the extraction from their rearing tube, and their
330 exposure to a new environment during behavioral tests.

331 The second behavior, ‘twisting’, consists of multiple and rapid torsions in response to
332 contact between a larva’s body and a parasitoid sting. We mimicked a parasitoid attack by
333 touching the dorsal part of the caterpillar with a fine brush, after 30 s of acclimation under the
334 lid of a Petri dish. Each individual was touched four times in 1 min. The entire sequence was
335 video recorded (HDR-CX220E, Sony) and further analyzed in slow motion with Kinovea
336 0.8.15 software to score the total number of torsions over the four touches.

337 Finally, we examined ‘dropping’ behavior, which describes how caterpillars faced
338 with a foraging parasitoid can drop and weave a silk thread to return to their living patch once

339 the parasitoid has left it. Larvae were installed at the top of a 150 cm high bracket. After an
340 acclimation of 30 s under a reversed Eppendorf tube, each larva was touched with a fine brush
341 (as described in the previous paragraph) until it fell. For caterpillars weaving a thread, we
342 measured its length with a ruler (precision ± 3 mm) after 5 s of stabilization. Thread length is
343 supposed to be positively linked to larval escape ability (Vogelweith et al., 2014).

344 Once the three behavioral tests were carried out, larvae were returned to their rearing
345 tube and associated thermal regime for 3 h before the immune measurements were taken. The
346 ability of the larvae to kill parasitoid eggs was estimated by measuring hemocyte
347 concentration and activity of the phenoloxidase (PO). Hemocytes are immune cells that freely
348 circulate in hemolymph of insects and bind to parasitoid eggs. Hemocytes release the PO
349 enzyme that catalyzes melanization reactions occurring around parasitoid eggs as part of the
350 immune response (Lavine and Strand, 2002). Caterpillars were not challenged prior to these
351 immune tests, because they naturally display high levels of basal immunity and do not
352 significantly respond to an infection (Vogelweith et al., 2014). Larvae were chilled on ice for
353 30 min and then photographed using a stereomicroscope at 12.5x magnification (Stemi 508,
354 Zeiss) to measure the distance between the most distant margins of the head capsule
355 (precision: ± 0.1 μm) as a reliable estimator of larval body size (Delbac et al., 2010). For this
356 experimental group, we measured larval body size rather than body mass since body size is
357 known to correlate with some of the larval defenses in this species (Iltis et al., 2018) and
358 because caterpillars cannot be accurately weighed while alive (Muller et al., 2015). One 2 μl
359 sample of hemolymph was then collected from a prick in the dorsal part of the abdomen with
360 a cold sterile glass capillary (Hirschmann Laborgeräte, Eberstadt). This sample was diluted in
361 20 μl of cold phosphate-buffered saline (PBS, 8.74 g NaCl, 1.78 g Na₂HPO₄, 1000 ml
362 distilled water, pH 6.5). A volume of 10 μl of this solution was immediately withdrawn to
363 estimate hemocyte concentration with a Neubauer improved hemocytometer under a phase

364 contrast microscope at 400x magnification (Primo Star, Zeiss). The remaining 12 μ l of diluted
365 hemolymph were supplemented with 10 μ l of PBS. These samples were frozen in liquid
366 nitrogen and stored at -80°C for the latter estimation of PO activity.

367 We distinguished between functional PO naturally activated in hemolymph (PO
368 activity) and total PO including functional PO and proenzymes (total PO activity). These
369 measurements were based on the spectrophotometry method described by Vogelweith et al.
370 (2013). Hemolymph samples were allowed to thaw very slowly on ice and centrifuged (4000
371 g, 15 min, 4°C) before 5 μ l of supernatant were transferred to microplate wells. These
372 samples were supplemented with either 160 μ l of diluted PBS solution (35 ml ultrapure water,
373 5 ml filtered PBS) for PO activity measurement, or with 160 μ l of chymotrypsin solution (35
374 ml ultrapure water, 5 ml filtered PBS, 2.45 mg trypsin) for total PO activity measurement.
375 Finally, 20 μ l of L-Dopa solution (40 ml ultrapure water, 160 mg L-Dopamine) were
376 deposited in each well as a colorimetric substrate. An enzymatic reaction proceeded for 40
377 min at 30°C in a microplate reader (Versamax, Molecular Devices), during which the
378 readings were taken every 15 s at 490 nm. Data were subsequently analyzed with the SOFT-
379 Max Pro 4.0 software. We recorded maximum enzyme activity (V_{max}), corresponding to the
380 slope of the absorbance curve during its linear phase. All immune assessments were reported
381 to 1 μ l of pure hemolymph.

382 *2.6 Statistical analyses*

383 Development time and pupal mass were normally distributed and displayed homogenous
384 variances (homoscedasticity) between thermal regimes and sexes. Consequently, they were
385 analyzed by means of two-way Analyses of Variance (ANOVAs) integrating thermal regime,
386 sex and their interaction. Survival and sex ratio were expressed in proportions and therefore
387 compared among thermal regimes with Generalized Linear Models (GLMs)-binomial error.

388 To control for allometric effects on larval physiological and behavioral traits, we included
389 either larval body mass (for energetic reserves) or larval body size (for defensive behaviors
390 and immunity) as a covariate in the statistical models, alongside thermal regime as an
391 explanatory variable. Most of the data were normally distributed (the reserves of the four
392 energetic compounds, hemocyte concentration, total PO activity) or required a logarithm
393 transformation to satisfy normality assumption (thread length and PO activity), and their
394 variances were homogenous between thermal regimes. Accordingly, all these data were
395 studied with analyses of covariance (ANCOVAs) testing for homogeneity of slopes and
396 intercepts, as these models integrated the effects of the explanatory variable (thermal regime),
397 the associated covariate (larval body mass or larval body size) and their interaction. As
398 overdispersed count data, the number of lines caterpillars crossed and the number of twists
399 were analyzed with GLM-negative binomial error. Normality and homoscedasticity were
400 assessed with a Shapiro-Wilk and a Levene test, respectively. For count data, overdispersion
401 (observed variance greater than the theoretical one predicted by the fitted model) was detected
402 with a postregression likelihood ratio test (Cameron and Trivedi, 1990). All statistical
403 analyses were carried out using R 3.5.2 software.

404 **3. Results**

405 *3.1 Group 1: development time, pupal mass, survival and sex ratio*

406 Neither development time (two-way ANOVA: $F_{1,832} = 0.04$, $P = 0.84$) nor pupal mass (two-
407 way ANOVA: $F_{1,832} = 0.39$, $P = 0.53$) was impacted by the interaction between thermal
408 regime and sex. Development time was modulated by thermal regime ($F_{1,832} = 639$, $P < 0.001$)
409 and sex ($F_{1,832} = 26.5$, $P < 0.001$) in an additive way: individuals reared in future, warmer
410 conditions needed less time to complete their larval development than those reared in current,
411 cooler conditions, and females developed slower than males (Table 2). Pupal mass was

412 affected by sex ($F_{1,832} = 1308, P < 0.001$) but not by thermal regime ($F_{1,832} = 0.67, P = 0.41$),
413 as females being significantly heavier than males (Table 2). Survival was influenced by
414 thermal regime (GLM-binomial error: $\chi_1^2 = 10.6, P < 0.001$): larvae reared in warmer
415 conditions were more prone to survive until the adult stage than those held in cooler
416 conditions (Table 2). Adult sex ratio did not differ between thermal regimes (GLM-binomial
417 error: $\chi_1^2 = 0.02, P = 0.88$) (Table 2).

418 **Table 2** Mean values and 95% confidence intervals of development time (time between egg hatching and pupation), pupal mass, survival (from
 419 larval to adult stage), and sex ratio (proportion of males in the adult population) for the two thermal regimes. Different letters indicate significant
 420 differences for a given trait ($P < 0.05$).

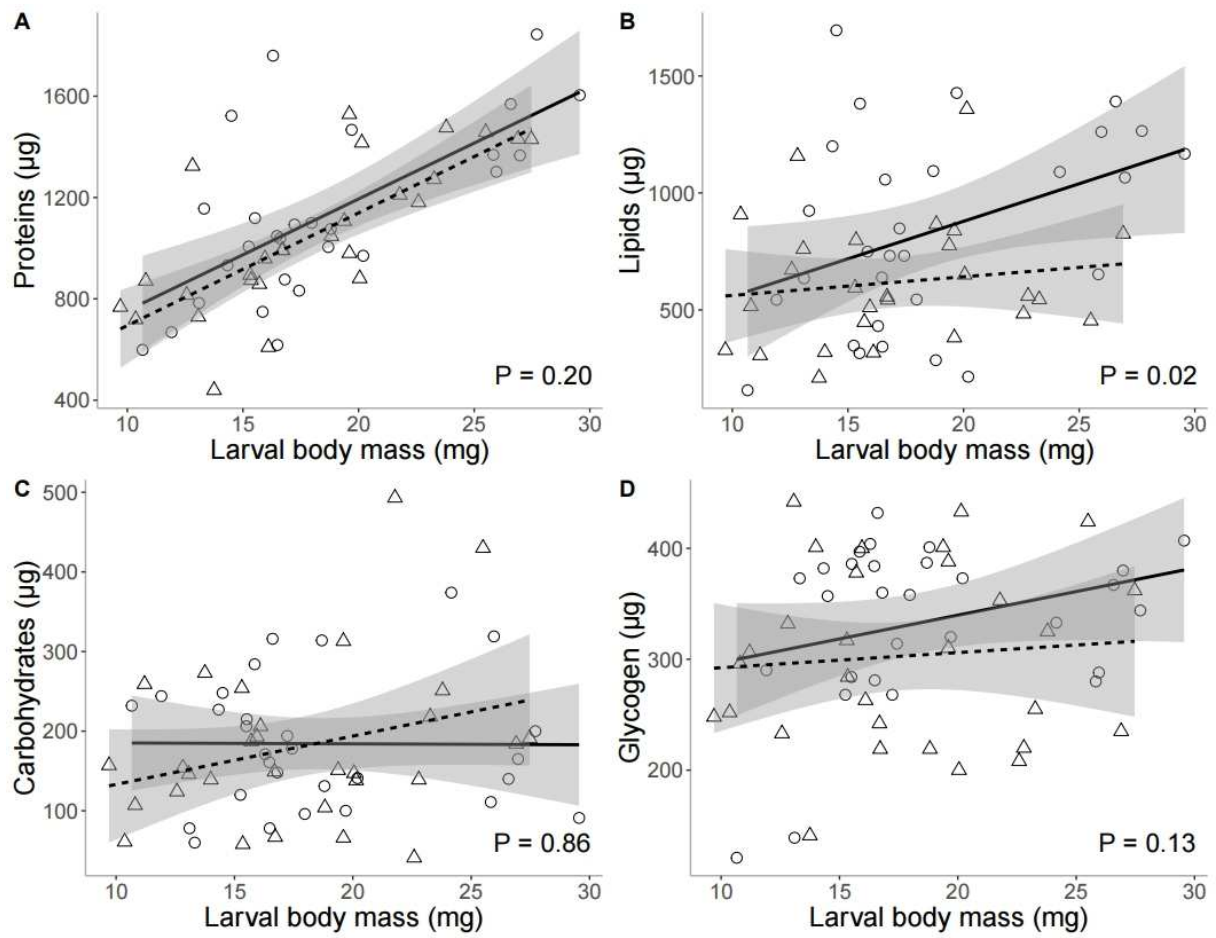
		Current regime	Future regime
Development time (days)	Males	24.6 [24.0; 25.2] ^a	16.9 [16.4; 17.3] ^b
	Females	26.3 [25.5; 27.0] ^c	18.4 [17.8; 19.0] ^d
Pupal mass (mg)	Males	9.00 [8.9; 9.2] ^a	9.00 [8.9; 9.1] ^a
	Females	12.8 [12.5; 13.1] ^b	12.7 [12.4; 12.9] ^b
Survival (%)		43.5 [38.4; 48.6] ^a	48.4 [43.9; 52.9] ^b
Sex ratio (%)		59.3 [54.2; 64.4]	58.5 [54.0; 63.0]

421 *3.2 Group 2: energetic assessments*

422 None of the four energetic compounds was affected by the interaction between thermal
423 regime and larval body mass (Table 3). Among these compounds, only the body amount of
424 lipids was influenced by thermal regime, while the amounts of proteins, soluble carbohydrates
425 and glycogen remained the same across thermal regimes (Table 3, Fig. 1A, B, C, D).
426 Caterpillars reared in warmer conditions stocked less lipid reserves at the end of their larval
427 development relative to their counterparts reared in cooler conditions (Fig. 1B). Protein and
428 lipid body amounts were both affected positively by larval body mass (Table 3, Fig. 1A, B):
429 heaviest larvae accumulated more reserves than lightest ones (proteins: slope = 44.1; lipids:
430 slope = 32.1).

431 **Table 3** Effects of thermal regime, larval body mass and their interaction on the total body amounts of the four energetic compounds. All data
 432 were studied with analyses of covariance testing for homogeneity of slopes and intercepts. Bold values indicate significant effects ($P < 0.05$).

	Thermal regime		Larval body mass		Regime * Body mass	
	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>
Proteins	$F_{1,50} = 1.72$	0.20	$F_{1,50} = 50.0$	< 0.001	$F_{1,50} = 0.001$	0.98
Lipids	$F_{1,52} = 5.69$	0.02	$F_{1,52} = 5.34$	0.02	$F_{1,52} = 1.63$	0.21
Soluble carbohydrates	$F_{1,55} = 0.03$	0.86	$F_{1,55} = 1.50$	0.23	$F_{1,55} = 1.64$	0.20
Glycogen	$F_{1,55} = 2.36$	0.13	$F_{1,55} = 1.98$	0.16	$F_{1,55} = 0.52$	0.47



433

434

Fig. 1 Effect of thermal regime (circles and solid lines: current regime, triangles and dashed

435

lines: future regime) in relation with larval fresh body mass on the total body amounts of

436

proteins (A), lipids (B), soluble carbohydrates (C), and glycogen (D). Shaded area around

437

each line corresponds to the 95% confidence interval for the predicted values of the linear

438

regression. Reported *P* values refer to the simple effect of thermal regime on each energetic

439

compound (testing homogeneity of intercepts between solid and dashed lines).

440 *3.3 Group 3: behavioral and immune defenses*

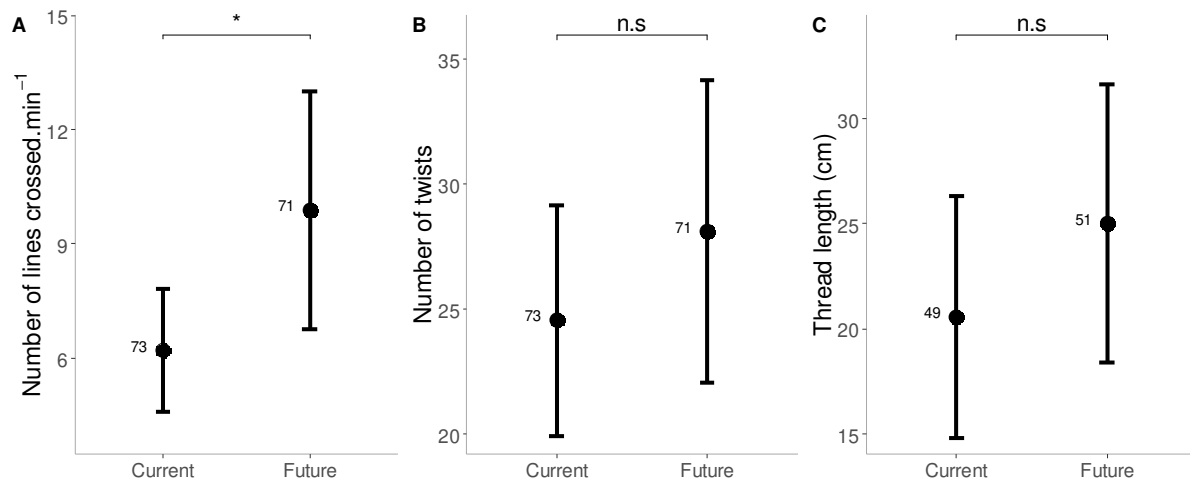
441 None of the behavioral or immune traits was impacted by the interaction between thermal
442 regime and larval body size (Table 4). Among the behavioral traits examined, only the
443 number of lines crossed by caterpillars during ‘flee’ tests was influenced by thermal regime:
444 larvae reared and tested at warmer conditions crossed significantly more lines relative to
445 individuals originating from cooler conditions (Table 4, Fig. 2A). Thermal regime did not
446 affect ‘twisting’ (number of twists) and ‘dropping’ (length of the silk thread woven) behaviors
447 (Table 4, Fig. 2B, C). From an immunological perspective, hemocyte concentration and PO
448 activity were not impacted by thermal regime (Table 4, Fig. 3A, B). However, total PO
449 activity was significantly modulated by thermal regime: larvae reared in warmer conditions
450 displayed lower levels of total PO activity compared with those held in colder conditions
451 (Table 4, Fig. 3C). None of the behavioral and immune traits was modulated by larval body
452 size (Table 4).

453 **Table 4** Effects of thermal regime, larval body size (head capsule width) and their interaction on different defensive behaviors and immune traits
 454 involved in defenses of caterpillars against larval parasitoids. Bold values indicate significant effects ($P < 0.05$).

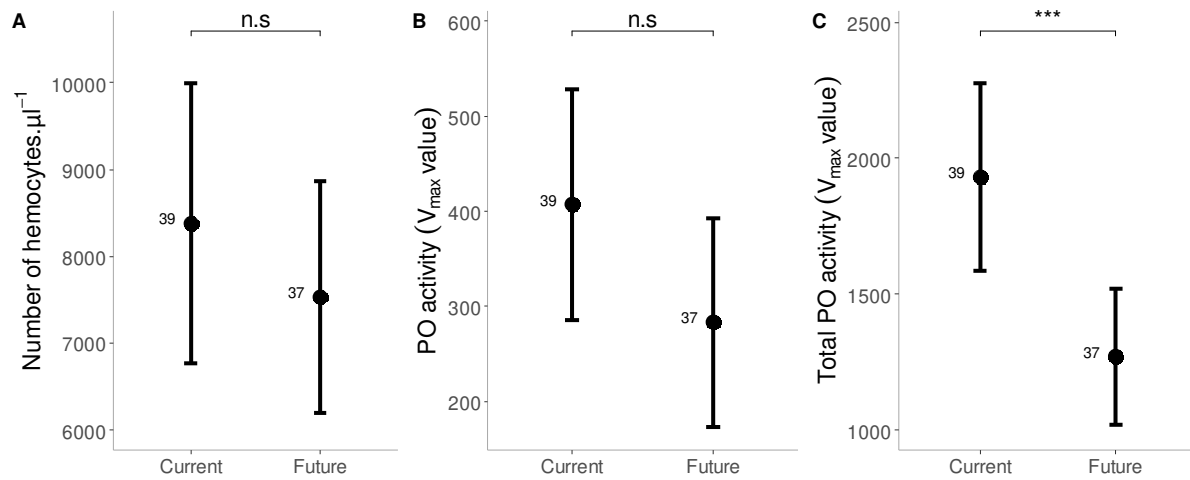
	Thermal regime		Larval body size		Regime * Body size	
	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>
<i>Defensive behaviors</i>						
Number of lines ^a	$\chi_1^2 = \mathbf{6.88}$	0.02	$\chi_1^2 = 0.24$	0.67	$\chi_1^2 = 0.50$	0.54
Number of twists ^a	$\chi_1^2 = 0.64$	0.35	$\chi_1^2 = 4.10^{-5}$	0.99	$\chi_1^2 = 0.80$	0.30
Thread length ^b	$F_{1,96} = 1.58$	0.21	$F_{1,96} = 1.59$	0.21	$F_{1,96} = 0.05$	0.82
<i>Immune traits</i>						
Hemocyte concentration ^b	$F_{1,72} = 0.64$	0.43	$F_{1,72} = 0.08$	0.78	$F_{1,72} = 0.67$	0.42
PO activity ^b	$F_{1,72} = 3.71$	0.06	$F_{1,72} = 0.89$	0.35	$F_{1,72} = 0.70$	0.41
Total PO activity ^b	$F_{1,72} = \mathbf{9.66}$	< 0.001	$F_{1,72} = 1.28$	0.26	$F_{1,72} = 0.39$	0.53

455 ^a Generalized linear model-negative binomial error

456 ^b Analysis of covariance



457
 458 **Fig. 2** Effect of thermal regime on the mean (\pm 95% c.i.) of the number of lines crossed by
 459 larvae during ‘flee’ tests (A), the number of twists over four touches (B), and the length of the
 460 silk thread spun (C). Asterisks highlight significant differences (*** $P < 0.001$, ** $P < 0.01$, *
 461 $P < 0.05$, n.s. non-significant) and numbers refer to sample sizes.



462
 463 **Fig. 3** Effect of thermal regime on the mean (\pm 95% c.i.) of hemocyte concentration (A),
 464 phenoloxidase activity (B), and total phenoloxidase activity (C). All these immune
 465 measurements were reported to 1 μl of pure hemolymph collected from fifth instar
 466 caterpillars. Asterisks highlight significant differences (*** $P < 0.001$, ** $P < 0.01$, * $P <$
 467 0.05, n.s. non-significant) and numbers refer to sample sizes.

468 **4. Discussion**

469 Within the framework of global warming, we here examined the responses of different
470 fitness-related traits in caterpillars of the moth *L. botrana* to realistic daily fluctuating regimes
471 reflecting current and future conditions in Eastern France. Many of the traits inspected were
472 affected by our experimental imitation of climate change. Warming positively influenced
473 caterpillars' development (leading to a 31% reduction in the time needed to complete all five
474 larval instars), survival from first instar larva to adult stage (+19%) and larval ability to
475 escape natural enemies through the expression of 'flee' behavior (+60%). Conversely, rising
476 temperatures led to depletion of lipid reserves (-26%) and reduced total PO activity (-34%).
477 Such a diversity of thermal responses among traits strengthens the importance of an
478 integrative approach involving measurements on a wide array of traits to accurately forecast
479 the consequences of global warming for an organism's performance, and the way it will
480 interact with adjacent trophic levels.

481 Some traits related with population dynamics were positively affected by rising
482 temperatures, presumably indicating an overall increase in caterpillars' performance under a
483 simulated global warming scenario. Indeed, caterpillars experiencing higher, future
484 temperatures developed faster and showed increased survival rates compared with individuals
485 kept under thermal conditions similar to those currently recorded in Eastern France. These
486 findings remain in accordance with previous studies showing that the thermal optimum for the
487 development of *L. botrana* larvae – including the laboratory strain used in this study – ranges
488 from 28°C to 30°C (Briere and Pracros, 1998; Iltis et al., 2018). Hence, the experimental
489 warming applied here (mean temperature increasing from 20.5 to 25.8°C) positively impacted
490 development rates by bringing environmental temperatures closer to the thermal optimum of
491 *L. botrana*. Similarly to a previous work conducted on the same species (Thiéry and Moreau,
492 2005), we found that faster development was accompanied with higher survival rates. One

493 plausible explanation to these results could involve a phenomenon known as ‘developmental
494 resistance’, whereby disease resistance usually increases throughout larval stages in some
495 butterfly species (McNeil et al., 2010; Shikano et al., 2018). Hence, caterpillars may benefit
496 from a faster development under warmer conditions by spending shorter in the early
497 ontogenetic stages, which are the most susceptible to mortality driven by pathogenic
498 infections (Valadez-Lira et al., 2012).

499 As a capital breeding species, we would have expected *L. botrana* individuals to incur
500 costs arising from a shrunk larval stage (i.e. a reduced time allowed for feeding) in warmer
501 conditions, especially regarding pupal mass. Contrary to these initial expectations, pupal mass
502 did not differ between thermal regimes. These results confirm that *L. botrana* caterpillars
503 could compensate for a shortened feeding period under hotter conditions through an increase
504 in daily gain of body mass (i.e. growth rate) (Iltis et al., 2018). The underlying mechanisms to
505 support a high growth rate in warm conditions could involve an increased food intake rate
506 and/or an enhanced efficiency at converting food into body matter when exposed to hotter
507 conditions (Bauerfeind and Fischer, 2013; Lee and Roh, 2010; Lemoine et al., 2014).
508 Nonetheless, the 26% decrease in the body amounts of lipids observed in warmer conditions
509 seems to indicate that larvae cannot compensate for the effects of warming on energetic
510 budgets, with costs being specifically apparent on fat reserves (the three other energetic
511 compounds remaining unaffected by temperature). Interestingly, previous work also recorded
512 a reduced lipid storage efficiency for fast developing caterpillars in *Spodoptera exigua*
513 (Lepidoptera: Noctuidae), suggesting that high development rates induced by warming may
514 be accompanied with a decline in lipid reserves (Lee and Roh, 2010). These findings indicate
515 that the lipid energetic compartment could be particularly susceptible to the detrimental
516 effects of a reduced feeding duration caused by an accelerated development under warmer
517 conditions.

518 Aside from traits related with population dynamics, thermal conditions modulated the
519 expression of several defensive traits involved in trophic interactions between *L. botrana*
520 caterpillars and their natural enemies, especially the guild of parasitoids. Concerning
521 behavioral traits, it is not possible to identify the mechanisms underlying the thermal response
522 of ‘flee’ behavior at this stage. It may arise from long-term effects, whereby temperature met
523 throughout larval development might have impacted the physiological state of caterpillars and
524 their resource partitioning between competing defensive traits (Abram et al., 2017). It could
525 also be attributable to short-term effects related with temperature prevailing during behavioral
526 tests, which might have influenced caterpillars’ metabolic rates and/or triggered
527 thermosensory processes leading to behavioral adjustments following perception of heat
528 conditions (Abram et al., 2017; Soto-Padilla et al., 2018). In terms of trophic dynamics, our
529 findings indicate that increasing temperatures should enhance the ability of *L. botrana*
530 caterpillars to escape from natural enemies through upregulation of ‘flee’ behavior. This does
531 not, however, necessarily imply a lower larval susceptibility to parasitism, provided that the
532 behaviors specifically expressed in case of an attempt of parasitoid oviposition (‘twisting’ and
533 ‘dropping’) remained unaffected by thermal conditions.

534 Concerning immune traits, total PO activity was the only immune trait affected
535 (negatively) by temperature, congruently with a previous work on *L. botrana* (Iltis et al.,
536 2018) and our hypothesis that global warming could induce immunosuppressive effects in
537 larvae of this species. Intriguingly, the decrease in total PO activity was not explained by a
538 concomitant lowering of hemocyte concentration, which remained constant across thermal
539 treatments in our study. Hemocyte populations are composed by different cell types (four
540 types have been identified in larvae of the moth *Eupoecilia ambiguella*, a relative of *L.*
541 *botrana*), each having a particular role in immunity (Lavine and Strand, 2002; Vogelweith et
542 al., 2016). In this regard, we could postulate that thermal conditions might influence the

543 relative abundances of hemocyte types in *L. botrana* larvae, tending to decrease the
544 proportion of cells involved in synthesis of PO zymogens (e.g. oenocytoids). Our findings
545 also indicate that, despite no effect on basal immunity (PO activity), warming could
546 significantly reduce the ability of *L. botrana* caterpillars to respond to a parasitic infection
547 because hotter rearing conditions decreased total PO activity. Indeed, the activation of PO
548 cascade system is required to catalyze melanization of parasitoid eggs (González-Santoyo and
549 Córdoba-Aguilar, 2012; Kanost and Gorman, 2008). However, it is worth noting that, in our
550 immunological assays, immune reactions were allowed to proceed at standard temperature.
551 Considering that, in lepidopteran species, the kinetics of PO enzyme will likely increase
552 alongside larval body temperature up to a certain limit (Xue et al., 2006), it remains possible
553 that larvae reared in warmer conditions invested less in the production of the active form of
554 PO as an adaptive choice to keep the overall enzymatic activity constant across temperatures.
555 Further insights into the functional consequences of this variation in larval immunity – for
556 instance by measuring the *in vivo* ability of caterpillars to encapsulate and melanize foreign
557 bodies at different temperatures – would be highly valuable to predict more thoroughly the
558 impacts of global warming on caterpillars' survival in context of high parasitism pressure as
559 recorded in vineyards.

560 **5. Conclusion**

561 In summary, our study brings new lines of evidence to suggest that multiple traits in a given
562 species may differ in their sensitivity to global warming, underlying complex consequences
563 for population dynamics and trophic interactions. Here, we found that, in terms of population
564 dynamics, warming brought *L. botrana* closer to its thermal optimum for larval development.
565 This should lead to a shorter generation time and therefore a higher future voltinism (number
566 of generations per growing season) of *L. botrana* in Eastern France, as recently observed in
567 meridional areas (Martín-Vertedor et al., 2010). To what extent these benefits might be

568 mitigated by the reduction of lipid reserves observed under warmer conditions remains not
569 well appreciated for now. A decline in lipids stock might, for instance, negatively impact the
570 survival of the naturally diapausing generation of *L. botrana*, because fat reserves are crucial
571 to survive extended non-feeding periods (Sinclair and Marshall, 2018). It may also persist into
572 the adult stage and adversely impact adult dispersal ability and reproduction (Vande Velde
573 and Van Dyck, 2013). Finally, we found that key traits involved in the trophic interactions
574 between *L. botrana* caterpillars and their larval parasitoids were altered by warming. At this
575 stage, predicting the consequences for future larval mortality induced by natural enemies in
576 agroecosystems is somewhat challenging, provided that the direction of the effect of warming
577 differed among defensive traits, and that parasitoids will also be impacted by rising
578 temperatures, from both physiological and behavioral perspectives (Hance et al., 2007).

579 Several considerations should, however, be carefully examined to provide a more
580 integrated picture of the impacts of warming for *L. botrana*, with respect to its evolutionary
581 and ecological context. First, our study specifically investigated the plastic response occurring
582 within one generation of *L. botrana* larvae exposed to an abrupt environmental warming. In
583 natural conditions climate disturbance will more likely translate into a gradual shift in thermal
584 conditions whose effects will spread across insect life stages and generations. For this reason,
585 it is now widely acknowledged that the response of insect species to global warming will not
586 solely be determined by intragenerational plasticity, but also by transgenerational plasticity
587 (whereby temperatures faced by one generation will influence the expression of a phenotype
588 in the subsequent generations) in concert with evolutionary processes (Donelson et al., 2018;
589 Sgrò et al., 2016). It would therefore be worth investigating whether these different types of
590 response might interact to shape the insect phenotype when experiencing gradual warming
591 over several successive generations. Second, to what extent the mosaic of microclimatic
592 conditions existing at the vine plant and grape bunch levels might create opportunities for

593 larval behavioral thermoregulation (through microsite selection) to alleviate the effects of
594 warming must be taken into consideration. Apparently homogenous landscapes are actually
595 composed of an important diversity of thermal conditions at much smaller, biologically
596 relevant spatial scale matching the body size of the insect (Potter et al., 2013; Terblanche et
597 al., 2015). Thus, variation in air temperature recorded in a given site at a macroclimatic scale
598 may bear little relationship to the variation in the operative body temperature of the insect
599 experiencing a set of contrasting microclimatic conditions. In vineyards, grape berry
600 temperature displays an important variability both between grape bunches and within a single
601 bunch, in relation with factors determining direct solar radiation (e.g. bunch exposure,
602 shading provided by leaf canopy), bunch compactness and architecture (Haselgrove et al.,
603 2000; Kiaeian Moosavi et al., 2018; Tello and Añez, 2017). Small scale variation in berry
604 temperature due to sunlight exposure has been shown to deeply impact *L. botrana*
605 performance, particularly in the early ontogenetic stages (egg hatching, larval settlement)
606 (Kiaeian Moosavi et al., 2018). Incorporating this natural thermal complexity at microhabitat
607 scales and the way it would be altered by a rapidly changing climatic context could therefore
608 become a next step towards a more thorough understanding of the consequences of global
609 warming for this insect pest.

610 **Competing interests**

611 The authors declare no conflict of interests.

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840



Global warming



Dijon



Vineyards

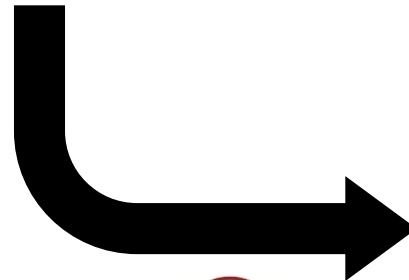
+ 5.3°C in mean temperature

+ 0.6°C in daily thermal range

Positive effect



Lobesia botrana



Negative effect

Development



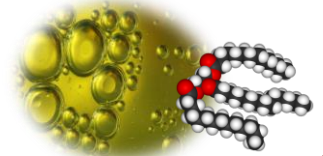
Survival



Movement speed



Lipid reserves



Immunity

