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1 **Thermal tolerance of two Diptera that pollinate thermogenic plants**

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8

9 **Abstract**

10 Pollinating insects can be exposed to temperatures far from ambient air when visiting flowers,
11 reducing their warming tolerance. Typically, such scenario occurs when flowers are exposed
12 to solar radiation. The case of thermogenic flowers is particular because they warm up even
13 when they are not exposed to solar energy. The flowers of *Arum* attract their pollinators with a
14 deceptive method and trap them for a whole day, thereby imposing elevated temperature to
15 visiting insects. Therefore, we predict a relatively high basal thermal tolerance in those
16 insects. The aim of this study was to assess the thermal tolerance and warming tolerance of
17 females of two fly species (genus *Psychoda*) pollinating *Arum* sp. (thermogenic plant). We
18 measured their critical temperature (CTmax) and its response to rate of temperature increase
19 as well as acclimation period to moderate temperature of 25°C. We found relatively low
20 CTmax (33.7°C on average) for both species, and a weak response to acclimation period and
21 ramping rate. In general, the thermal tolerance increased with a rapid ramping in temperature.
22 To evaluate the warming tolerance, we compared thermal tolerance limits to flower
23 temperatures measured in the field. We highlighted that the temperature of the thermogenic

24 floral organ could reach values close to the thermal tolerance threshold of pollinators. This
25 discovery raises questions about the sustainability of the interaction between these
26 thermogenic plants and their pollinators.

27 Key words

28 *Psychoda*; Critical Thermal maximum; plasticity; heat tolerance; thermal limit; Wild
29 pollinator.

30 1. Introduction

31 Ectotherms depend on the heat in their environment and a multitude of biological traits (e.g.
32 growth, size, lifespan, reproduction) is governed by their body temperature (Angilletta, 2009;
33 Pincebourde et al., 2017). As a consequence, the thermal niche of ectotherms is constrained
34 by temperature patterns, and to a large extent by the maximal temperature they can sustain
35 (Hoffmann, 2010; Santos et al., 2011). Thermal tolerance limits are extensively used by
36 macroecologists to infer the vulnerability of ectotherms to environmental issues including
37 climate change (Pinsky et al., 2019; Sunday et al., 2011). Indeed, the past decade has seen the
38 emergence of a compiled dataset of thermal tolerance limits across animals that is now widely
39 used to estimate their vulnerability across realms and geographical extents (Bennett et al.,
40 2018; Sunday et al., 2011), and to infer the potential response of species distributions to
41 climate change (Sunday et al., 2012). However, the use of thermal tolerance limits, such as the
42 CTmax (maximal critical temperature), in this context should be done with caution especially
43 when we consider the variability of this trait (Clusella-Trullas et al., 2021). The inter- and
44 intra-specific variability of ectotherms' thermal tolerance has become a hot topic in global
45 change biology and conservation biology.

46

47 At the inter-specific level, the variance of thermal tolerance limits is considerable for a given
48 latitude (Sunday et al., 2011). This variability can be explained by both adaptation to current
49 extreme temperatures and by phylogenetic constraints (Bennett et al., 2021). Local adaptation
50 of thermal limits can occur at very fine spatial scale, in response to the microclimatic patterns
51 such as a single leaf temperature pattern (Pincebourde and Casas, 2015). At the intra-specific
52 level, thermal tolerance limits of ectotherms vary according to their own plasticity (Ma et al.,
53 2021) but also in relation to methodological issues (Terblanche et al., 2007). The maximum
54 critical temperature (CT_{max}) is one of the most common metrics used to inform on a species
55 thermal tolerance limit (Angilletta, 2009; Huey and Stevenson, 1979). The CT_{max} is
56 described as the temperature at which the ectotherm loses the control of its mobility under
57 controlled warming conditions (Kingsolver and Umbanhowar, 2018). This loss of locomotor
58 coordination can be characterized by a stop of locomotion, a knockdown or a particular flight
59 behavior in some flying insects (Lutterschmidt and Hutchison, 1997). The estimation of
60 critical thermal limits can be achieved using a dynamic assay (Jørgensen et al., 2021, 2019;
61 Rezende et al., 2020; Terblanche et al., 2011, 2007). In this dynamic method, both the
62 temperature at the start of the experiment and the rise in temperature influence the CT_{max} of
63 individuals (Kingsolver and Umbanhowar, 2018). For instance, a steeper temperature ramping
64 often results in a higher CT_{max} than a slow temperature increase, which accumulates stress
65 during the slow warming (Terblanche et al., 2007). Those methodological considerations
66 require the CT_{max} to be measured at several ramping values or using particular
67 methodologies (Jørgensen et al., 2019) and to be compared to estimate the realized range of
68 CT_{max} values.

69

70 Beyond the assessment method, the thermal tolerance limits of organisms are modulated in
71 response to environmental factors, including starvation (Terblanche et al., 2011), exposure to

72 pollutants, or acclimation (Dinh et al., 2016). Basically, the local adaptation hypothesis
73 suggests that the thermal tolerance of organisms exposed to hot environments is higher than
74 organisms from relatively colder habitats (MacLean et al., 2016; Sunday et al., 2012).
75 However, acclimation to low or high temperatures is certainly one of the most plastic
76 mechanisms with beneficial effects for survival (Willot et al., 2021). In general, acclimation
77 to elevated temperature enhances the CTmax (Kingsolver and Umbanhowar, 2018; Pandey et
78 al., 2021), in line with mechanisms of heat hardening (Sørensen et al., 2019). The acclimation
79 time also plays a crucial role in the hardening process and a longer acclimation time favors
80 higher CTmax (Peck et al., 2014), although this effect depends directly on the choice of
81 acclimation temperature and duration relative to the basal thermal tolerance limit. However,
82 these acclimation capacities are not equal across species and could depend on the basal
83 CTmax (Barley et al., 2021; Gunderson et al., 2017).

84

85 The issues above about the variability of CTmax are partially integrated into the most
86 complete database of thermal limits for ectotherms (Bennett et al., 2018). Insects, which
87 represent the vast majority of biodiversity on earth (Bar-On et al., 2018), are well represented
88 in this database but with a strong bias towards ants and herbivore insects. Pollinator insects
89 are represented mostly by bees, wasps and drosophila, thereby highlighting the gap in our
90 knowledge on thermal limits of wild pollinators including other Dipteran species. Indeed, the
91 order Dipteran is the second most important group of flower pollinators in the world (Larson
92 et al., 2001). Wild pollinators are playing a crucial role in the ecosystem service of pollination
93 of wild and cultivated plants (Garibaldi et al., 2013), and determining their thermal limits
94 should help to anticipate their breakdown as the climate continues to warm up.

95

96 Here we focus on insect pollinators for which the thermal biology remains totally overlooked:
97 flies of the genus *Psychoda* (Diptera), also called moth flies. Several species in this group are
98 visitors for flowers of *Arum* (*Arum maculatum* and *Arum italicum*) in Western Europe (Kite et
99 al., 1998). The two species of *Psychoda* (*Psychoda sigma* and *Psychoda uncinula*) are
100 cosmopolitan. These moth flies are found in relatively humid environments to carry out the
101 larval part of their life cycle (Laurence, 1954; Speirs et al., 2020). Indeed, the larvae in the
102 genus *Psychoda* are semi aquatic (e.g. *P. alternata* that is found in pipes) and feed on
103 decomposing organic material (Satchell, 1947a). Although the biology of these moth flies has
104 been neglected, the pollination system was intensively studied. The flower of *Arum* attracts
105 these insects by a deceptive method. This means that the plant provides no apparent benefit
106 during the pollination process (Chartier et al., 2013) (but see Gibernau et al., 2004 for a
107 discussion on rewards). The other peculiarity of *Arum* flowers is that they produce heat during
108 anthesis (i.e. thermogenesis; Marotz-Clausen et al., 2018). The structure of the flower and its
109 floral chamber ensures that pollinators remain trapped for a day in the flower (Bermadinger-
110 Stabentheiner and Stabentheiner, 1995). Thus, moth flies are subjected to the floral
111 temperature which can reach 30°C (M. Leclerc, pers. obs.) and be about 10°C warmer than
112 ambient air (Albre et al., 2003) in particular the afternoon. For this study we hypothesized that
113 pollinators would visit several flowers consecutively. Hence, based on the local adaptation
114 hypothesis (MacLean et al., 2016), one can expect higher CTmax in those species exposed to
115 a warm micro-environment in the floral chamber compared to insects living in open-air all
116 time.

117

118 The general objective of this work was to gather knowledge on the thermal biology of two
119 species of *Psychoda*, which are pollinators of *Arum* flowers. We determined the variation in
120 CTmax in relation to the duration of acclimation before the measurement and to the rate of

121 temperature increase during a dynamic (ramping) procedure. We expected an impact of
122 acclimation and rate in temperature increase consistent with previous studies (Sørensen et al.,
123 2019; Terblanche et al., 2011). More precisely, an increase in the acclimation duration should
124 promote the thermal tolerance limits by heat hardening mechanisms. In the same way, a faster
125 rate in temperature increase should generate higher thermal tolerance limits. Moreover, the
126 two species studied here visit the same ‘hot’ flowers. As such, a difference of thermal
127 tolerance between the two species was not necessarily expected, particularly given that they
128 belong to the same genus (phylogenetic constraint on the divergence of the CT_{max})
129 (Hoffmann et al., 2013). Overall, we anticipated relatively high thermal tolerance limits in
130 these species, compared to most temperate insects, given the elevated temperatures they
131 encounter within the flowers. Finally, we put into ecological perspective these CT_{max} values
132 by comparing the CT_{max} of the two flies with flower temperature patterns measured at our
133 study site. We assessed the extent to which those pollinators are close to their thermal
134 tolerance limits when visiting these hot flowers.

135

136 2. Materials methods

137 2.1 Biological material

138 The individuals we used in our experiments came from a laboratory rearing (>10 generations)
139 that was initialized from flies collected in floral chambers of *Arum italicum* in April 2020 near
140 Tours, France (47°22'54.4"N, 0°50'07.5"E). The rearing was held in a climatic chamber at
141 20°C and photoperiod 10:14 (light-dark) (adapted in our laboratory from Redborg et al.,
142 1983). The flies were reared in containers (glass jars with dimension 6.5 x 6.5 x 12.5 cm) on a
143 substrate composed of horse dung and dechlorinated water. The substrate was changed every
144 month so that the nutritional quality was maintained across generations. The substrate was

145 hydrated twice a week to maintain a semi-aquatic medium for larvae. We focused on females
146 for the experiments below because mostly females visit the *Arum* flowers (Laina et al., 2022;
147 Szenteczki et al., 2020).

148 2.2 Experimental design:

149 The temperature in floral chambers of *Arum italicum* was measured for two purposes: (i) to
150 set biologically relevant acclimation and start temperatures for the ramping assay (see below),
151 and (ii) to compare the CTmax of the flies to the temperature they experience when visiting
152 the flowers. *Arum italicum* produces four peaks of thermogenesis and three are associated
153 with the male organ of the flower, which is present in the floral chamber (Albre et al., 2003).
154 We focused exclusively on the maximum temperature of the fourth peak because it occurs
155 when the moth flies are present (trapped) in the flower chamber (Lack and Diaz, 1991). Right
156 before anthesis, we equipped 21 different flowers with thermocouples connected to the
157 HOBO Onset 4 channel thermocouple logger (UX120-014M). One thermocouple was inserted
158 into the male part through the integument of the floral chamber while another thermocouple
159 was held in the open air near the flower, under the shade, to measure air temperature. The
160 loggers were set to record temperature every second. The flowers were sampled between
161 April 12th and May 18th 2021 at the same site that was used for the collection of insects.

162

163 We tested the combined effect of acclimation time at moderate (sublethal) temperature and
164 rate in temperature increase during the experiment on the CTmax on females of both
165 *Psychoda* species. Measurements of CTmax were performed on adult female *Psychoda* sp.
166 using a heating system with a Peltier module connected to a manual temperature control
167 station with a precision of 0.1°C. The use of a Peltier system made it possible to control
168 precisely the temperature of the set-up. We placed on the Peltier a block of aluminum dug

169 with 9 wells (capacity of 0.3 mL) to receive individual insects. The wells were covered with a
170 transparent plexiglass plate to prevent the insects from leaving and to be able to observe them.
171 The aluminum block was separated from the Peltier module with a copper plate covered on
172 both sides with a layer of thermal paste. A temperature probe connected to the control station
173 was inserted between the copper plate and the aluminum block. In addition, we controlled the
174 actual temperature in the wells and inside the block with two thermocouples connected to a
175 HOBO Onset 4 channel thermocouple logger (UX120-014M). The temperature corresponding
176 to the CTmax was taken from the reading of HOBO thermocouple in the well when we
177 observed a knockdown of the insect (e.g. disorganized spiral flight or complete loss of
178 mobility). We had 2 or 3 runs for each treatment with 6 to 8 individuals for each run. In total,
179 we measured the CTmax for 131 *P. sigma* and 117 *P. uncinula*. The room temperature was
180 constant at 25°C throughout the experiments with a relative humidity of about 50%.

181

182 For the acclimation procedure, we exposed the individuals of *Psychoda* sp. to a moderate
183 temperature (25°C) during different times (0, 15, 90, or 180 min) right before the
184 measurement of CTmax, thereby simulating roughly the visit of a flower (see below; mean
185 temperature was 26°C). To acclimate insects at 25°C, we placed the flies individually in
186 perforated tubes of 2mL in a climatic chamber set at 25°C under light. These treatments of
187 acclimation were crossed with different rates of temperature change (ramping). We tested the
188 impact of rise in temperature on the thermal limit of moth flies by varying the slope of the
189 temperature increase (0.1°C.min⁻¹ or 1°C.min⁻¹). Throughout the experiment, the slope for the
190 two ramps of the CTmax had a respective average rate of 0.10 ± 0.01 °C.min⁻¹ and 1.00 ±
191 0.04 °C.min⁻¹ (Mean ± standard deviation). The starting temperature was 25°C for all runs,
192 corresponding to the acclimation temperature. Indeed, Jorgensen et al. recommended using
193 the average maximum temperature of the hottest month as the starting temperature (Jørgensen

194 et al., 2019). Based on temperature readings in the collection area, the mean maximum
195 temperature for the hottest month was 24°C (June 2021).

196 2.3 Statistical analyses

197 The raw data presented a log-negative distribution (skewness of -1.6; calculated with function
198 *skewness* of package moments (0.14 version)). Therefore, they were transformed using the
199 following equation: $(\log_{10}(\max(\text{CTmax}+1) - \text{CTmax}))$ to normalized the data and to match
200 the requirements for parametric tests (determine graphically using the plots of the model). We
201 tested the impact of three factors: species, acclimation time and rate of temperature increase,
202 and their interactions, on the CTmax of the female *Psychoda* with an analysis of variance
203 (ANOVA) in the software R (version 4.0.3; R Development Core Team, 2020). We had a
204 significant interaction between these three factors. Therefore, we decomposed the model
205 according to the species and we reused ANOVA model considering only the factors of
206 acclimation and rate of temperature increase. We used the function *emmeans* of package
207 *emmeans* (1.5.2-1 version) to make pairwise comparisons on means. A correlation analysis
208 between air temperature and flower temperature was made using a Pearson correlation test.
209 We fixed the level of significance using a probability threshold of 0.05.

210 3. Results

211 3.1 Flower temperature

212 The ecological relevance of thermal biology traits can be assessed by comparing them to the
213 temperatures actually experienced by species in their environment. We found that the floral
214 temperature was always warmer than the ambient air in *A. italicum* (Figure 1). The
215 temperature experienced by the *Psychoda* sp. when visiting the flowers averaged $26.0 \pm 2.7^{\circ}\text{C}$
216 (Mean \pm standard deviation) for the 21 flowers. The flower temperature was correlated
217 positively to ambient air temperature (Spearman correlation: 0.57; $p < 0.01$). One flower

218 displayed the maximal temperature of 34.5°C. Globally, flower temperature increased with
219 ambient air temperature but the slope is much lower than 1.

220 3.2 Thermal tolerance of *Psychoda* species

221 The mean thermal tolerance values across all treatments ranged from 32.0°C to 35.4°C for *P.*
222 *sigma* and from 31.2 to 35.5 for *P. uncinula* (Figure 2). We obtained an interaction between
223 *Psychoda* species, acclimation time and rate of temperature increase that influenced the
224 CTmax ($F_{15,237} = 17.81$, $p < 0.001$ and $r^2 = 0.52$ – Table S1). When we decomposed the
225 model by the species factor, we observed different results for *P. sigma* and *P. uncinula* (Table
226 1). However, the CTmax values remained similar for the two species of *Psychoda*.

227

228 For *P. sigma*, acclimation time and rate of temperature increase modified the CTmax (Table
229 1). An exposure to a moderate temperature (25°C) before measuring the CTmax influenced its
230 thermal tolerance (Table 1). We showed a decrease of CTmax when females were exposed for
231 15 and 180 min at 25°C (Figure 2A). The rate of temperature increase had a strong impact on
232 the CTmax (Table 1). The CTmax was lower when the rate of temperature increase was low
233 (0.1°C.min⁻¹) compared to a slope of 1°C.min⁻¹ (overall means: 33.1°C and 34.7°C,
234 respectively).

235

236 For *P. uncinula*, we showed a significant impact of interaction between the rate of
237 temperature increase and acclimation time (Table 1). The CTmax of females decreased when
238 they were exposed at 25°C (independently of acclimation time) with a rate of 1°C.min⁻¹, while
239 the CTmax was the same for all acclimation times with a rate of 0.1°C.min⁻¹. Moreover, the
240 CTmax was lower with a rate of 0.1°C.min⁻¹ (Figure 2B).

241

242 Table 1: Statistic outputs of the ANOVA model on log-negative of CTmax as function of the
 243 rate of temperature increase (0.1 and 1°C.min⁻¹) and acclimation time (0, 15, 90, 180 min) of
 244 *P. sigma* and *P. uncinula*. The statistics of the model are respectively $F_{4,126} = 41.49$, $p <$
 245 0.001 , $r^2 = 0.55$ and $F_{7,109} = 24.53$, $p < 0.001$ and $r^2 = 0.59$

Species	Source	Df	Mean Square	F-ratio	P-value
<i>P. sigma</i>	Rate	1	0.8607	92.95	<0.001
	Acclimation time	3	0.2253	24.33	<0.001
	Error	126	1.1667		
<i>P. uncinula</i>	Rate	1	1.6450	140.330	<0.001
	Acclimation time	3	0.0284	2.419	0.07
	Rate:Acclimation time	3	0.0943	8.048	<0.001
	Error	109	0.0117		

246 4. Discussion:

247 Information on the thermal tolerance of ectotherms is extremely useful when assessing their
 248 potential vulnerability to environmental hazards including climate change (Clusella-Trullas et
 249 al., 2021). This knowledge is now accessible for a limited number of species (Bennett et al.,
 250 2018) relative to global number of species inhabiting Earth. In particular, among insects, this
 251 database is strongly biased towards rather large species. However, the body size distribution
 252 of biodiversity is asymmetric and peaks at a size of around 1 cm (May, 1988). This
 253 asymmetry encourages us to study the smallest creatures, which are under sampled
 254 (Kozłowski and Gawelczyk, 2002), including these *Psychoda* sp. Near nothing is known on
 255 the thermal biology of *Psychoda* species. The only studies available date back to the middle
 256 of 20th century (Biever and Mulla, 1966; Redborg et al., 1983; Satchell, 1947b; Solbe and
 257 Tozer, 1971), on a species of non-pollinating *Psychoda* (*P. alternata*). Species of moth flies
 258 are very atypical because they are subjected to the temperature of the flowers that they visit
 259 daily (pollination events). Contrary to our expectations, our results show that these insects,

260 which visit flowers that are warmer than ambient air, have a rather low CTmax in general. In
261 addition, the modulation of their CTmax is very limited, at least for the acclimation time at
262 25°C and the rate of temperature increase. This result seems to contradict the general idea
263 that the CTmax is more plastic when it is low basally (the trade-off hypothesis of thermal
264 tolerance plasticity; (Somero, 2010; Stillman, 2003)), joining a global analysis demonstrating
265 that level of plasticity of CTmax may not depend on the basal CTmax (Gunderson and
266 Stillman, 2015). However, a non-negligible modulation of heat thermal tolerance is found in
267 *Psychoda* flies in response to changing the rate of temperature increase and the duration of
268 acclimation procedure.

269

270 As expected, the CTmax of *Psychoda* is primarily altered by the rate of temperature increase
271 in the ramping assay. Usually, it is recommended to use rates close to those encountered in
272 natural environments, most often between 0.1°C and 0.25°C (Terblanche et al., 2007). The
273 choice of a high rate of temperature increase inevitably produces a high CTmax value
274 (Kingsolver and Umbanhowar, 2018). Indeed, the organisms experiencing a rapid rate in
275 temperature increase also spend less time at stressful sublethal temperatures. Therefore, they
276 accumulate less thermal stress during the same amount of time than individuals under a slow
277 warming rate. However, moth flies are subject to fast temperature variations when they arrive
278 in a hot flower of *Arum* (Albre et al., 2003). Indeed, the temperature of the flower can be up to
279 15°C higher than the ambient air when the pollinators enter the flower. They are subject to an
280 increase of 10 to 15°C within a very short time (a few minutes depending on their behavior;
281 M. Leclerc, pers. obs.). Therefore, the highest CTmax values obtained with the higher rate of
282 temperature increase certainly is more relevant in this pollination system. We may expect
283 *Psychoda* to be slightly more tolerant to high temperatures when they successively visit
284 several flowers with high temperatures. On the other hand, a fast warming rate may limit the

285 possibility for acclimation or other plastic responses that could help to tolerate high
286 temperatures (Ma et al., 2021).

287

288 The acclimation time at a moderate temperature (25°C) mattered for the CT_{max} to a lesser
289 extent than the rate of temperature increase. Moth flies frequently encounter this temperature
290 in flowers even if ambient air is well below 20°C (see Figure 2; (Albre et al., 2003)). Often,
291 short term acclimation to moderate or sublethal temperatures is thought to benefit the insect
292 since it allows to develop heat hardening process, increasing thereby the CT_{max} (Sgrò et al.,
293 2016; Willot et al., 2021). In *Psychoda*, acclimated females (15 and 180 min at 25°C) had a
294 lower CT_{max} than the non-acclimated individuals (control) in *P. sigma*. Acclimation had no
295 effect in *P. uncinula* females. The acclimation temperature may already be above the
296 optimum temperature for these organisms (Sinclair et al., 2016) and as long as this optimum is
297 not quantified, we cannot exclude that exposure to 25°C even for a short time could already
298 cause thermal stress accumulation. Nevertheless, short-term acclimation to temperatures
299 (37°C) close to basal CT_{max} (about 41°C) was still able to generate an increase in the CT_{max}
300 of drosophila flies (Sgrò et al., 2010). Similarly, 4 h exposure to 35°C induced an increase in
301 thermal tolerance in *Liriomyza* flies which display thermal limits around 40°C (Huang et al.,
302 2007). However, we measured the thermal tolerance of *Psychoda* directly after acclimation
303 without recovery time, contrasting to most designs applied to study the effect of acclimation
304 on thermal tolerance – we did this to simulate situation in flowers. It is therefore possible that
305 the lack of recovery time impeded *Psychoda* flies to mount up a physiological response
306 underlying hardening. Therefore, we cannot exclude that the *Psychoda* fly, if it survives the
307 exposure to high temperature in the first visited flower, would develop heat hardening
308 response for the next visited flower the day after. The quantification of HSPs (Heat Shock
309 Proteins) in acclimated versus non-acclimated individuals at different times after exposure to

310 heat would inform on their ability to initiate this hardening response (Huang et al., 2007; Ma
311 et al., 2021).

312

313 The warming tolerance of organisms (difference between the temperature in the habitat and
314 the upper thermal limit) is often used to refer to the vulnerability of species to climate change
315 (Clusella-Trullas et al., 2021). The thermal landscape of moth flies is rather extensive and
316 ranges from the cool forest litter to warm thermogenic plants that can reach 30°C. Thus, when
317 individuals of *P. sigma* are within the flowers, their warming tolerance is reduced (Table 1;
318 Figure 2). This warming tolerance observed in *Psychoda* is consistent with data already
319 inferred for most ectotherms (Sunday et al., 2014). However, one flower displayed a floral
320 temperature higher than the CTmax of the mothflies we studied here (Figure 1). Larger
321 sampling of flower temperatures could be considered to determine if it was a flower with an
322 isolated heating mechanism or if this pattern that is dangerous for the mothflies is more
323 common than expected. Nevertheless, in a context of global changes and an increase in the
324 intensity and frequency of heat waves, we can hypothesize that flower temperatures would be
325 even warmer as ambient air increases. One consequence might be that pollinators would be
326 exposed to temperatures closer to their upper thermal limit. The impact of environmental
327 change on the thermogenesis ability of *Arum* should be studied to assess this hypothesis.

328

329 The first and apparently the only thermal biology studies on *Psychoda* date from about 60
330 years ago (Biever and Mulla, 1966). Our study is the very first to provide knowledge on moth
331 flies that pollinate *Arum* flowers. The thermal environment of those flies when they are
332 trapped in the flower during almost an entire day is particularly different from ambient
333 conditions. The thermogenic flower exposes its pollinator to temperatures that are around 25-

334 27°C, sometimes higher. Therefore we expected the moth flies to be adapted to this
335 microenvironment, involving selection of the most heat tolerant individuals across generations
336 (Clusella-Trullas et al., 2021; Urban et al., 2016). Our result somehow contradicts this
337 hypothesis because the CTmax values that we report are several degrees lower than most
338 temperate insects (when comparing with values in the database GlobTherm; or when looking
339 at a subset of data for insects in Sunday et al., 2011; (Bennett et al., 2018)). Indeed, the
340 CTmax ranges from 29.5°C to 56°C (N=71 data; mean 36.3°C) for arthropods under
341 temperate latitudes (Pincebourde and Casas, 2019; Sunday et al., 2011). Our prediction was
342 based on the fact that pollinators would visit several flowers consecutively, thereby spending
343 almost their entire adult lifetime within flowers. However, mothflies may develop an aversion
344 to flowers might develop after this deceptive experience (deception by the plant), making
345 them exposed to such temperature only once in their life. The density of flowers in the locality
346 of the insects should also matter by modifying the encounter rate. Another (non-mutually
347 exclusive) explanation could be that these two *Psychoda* are living in the leaf litter and the
348 females normally lay eggs on decomposing organic matter (in dung or in decomposing plant
349 material in the litter). This litter environment is particularly buffered from temperature
350 extremes (Geiger, 1965) which could select for low basal heat tolerance in those species.
351 However, forest canopies are suffering from climate warming, leading to the opening of
352 canopies and to the increase in litter and soil temperatures (Allen et al., 2015; Zellweger et al.,
353 2020). Looking at the floral temperature in this context could be interesting to determine the
354 warming tolerance of these pollinators. We can hypothesize that the warming of the forest
355 floor causes an increase in floral temperature, thus reducing the warming tolerance. Finally,
356 we obviously lack critical data before we can pretend to estimate the vulnerability of
357 *Psychoda* species to climate change and the sustainability of the interaction between *Arum*
358 and their pollinator in this context.

359 Author contributions

360 Conceptualization and experimental design: ML, SP; experiments and monitoring: LG, ML;
361 Data and statistical analyses: ML; Original draft writing and preparation: ML, SP; Review
362 and final editing: ML, CRL, SP.

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365 Declaration of competing interest

366 The authors declare no conflict of interest.

367 Data availability

368 Data will be made available on request.

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375 Appendix A. Supplementary data

376 Supplementary data to this article can be found online at
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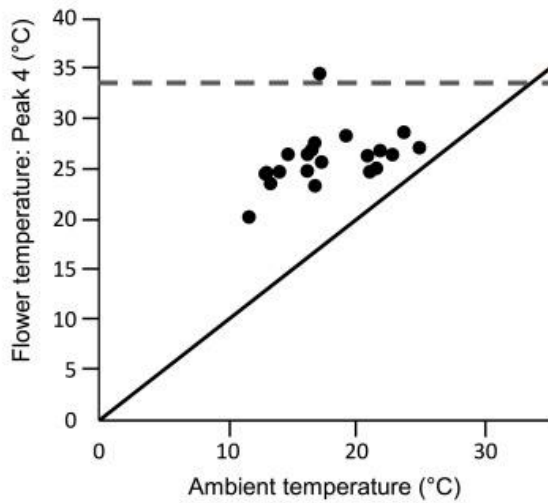
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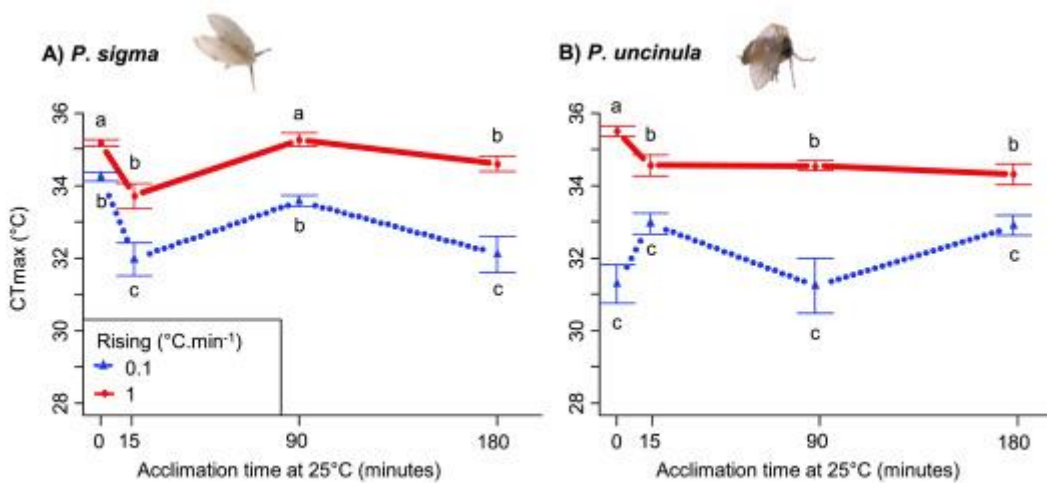
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588 Legend of figures



589

590 Figure 1: Plot showing the maximum temperature of the fourth temperature peak of *Arum*
 591 flowers (dots) as a function of the ambient temperature (near the flower). The line represents
 592 the 1:1 line, where the temperature of the flower would be equal to air temperature. The
 593 dotted horizontal line represents the mean of CTmax of two species of *Psychoda* in this study.



594

595 Figure 2: Thermal tolerance limits (CTmax) of the two species as a function of acclimation
 596 time (at 25°C) and the rate of temperature increase during the ramping, for (A) *P. sigma*
 597 (n=131) and (B) *P. uncinula* (n=117). Points are means \pm standard error. Significant
 598 differences ($p < 0.05$) in the Post-hoc test (Package *emmeans* version 1.5.1-1) are shown with
 599 different letters on the graphs.