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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 (CT<sub>max</sub>) 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 CT<sub>max</sub> (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<sub>max</sub>) 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<sub>max</sub> 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<sub>max</sub> of  
63 individuals (Kingsolver and Umbanhowar, 2018). For instance, a steeper temperature ramping  
64 often results in a higher CT<sub>max</sub> than a slow temperature increase, which accumulates stress  
65 during the slow warming (Terblanche et al., 2007). Those methodological considerations  
66 require the CT<sub>max</sub> 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<sub>max</sub> 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 CTmax)  
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 CTmax values  
132 by comparing the CTmax 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 12<sup>th</sup> and May 18<sup>th</sup> 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<sup>-1</sup> or 1°C.min<sup>-1</sup>). 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<sup>-1</sup> and 1.00 ±  
191 0.04 °C.min<sup>-1</sup> (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<sup>-1</sup>) compared to a slope of 1°C.min<sup>-1</sup> (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<sup>-1</sup>, while  
239 the CTmax was the same for all acclimation times with a rate of 0.1°C.min<sup>-1</sup>. Moreover, the  
240 CTmax was lower with a rate of 0.1°C.min<sup>-1</sup> (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<sup>-1</sup>) 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 20<sup>th</sup> 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<sub>max</sub> 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<sub>max</sub> (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<sub>max</sub> 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<sub>max</sub> (about 41°C) was still able to generate an increase in the CT<sub>max</sub>  
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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378

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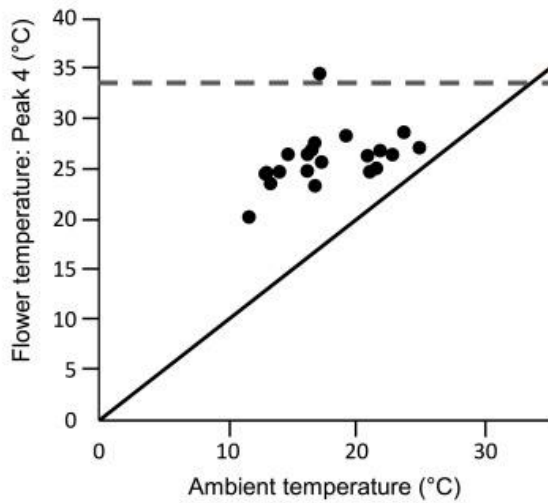
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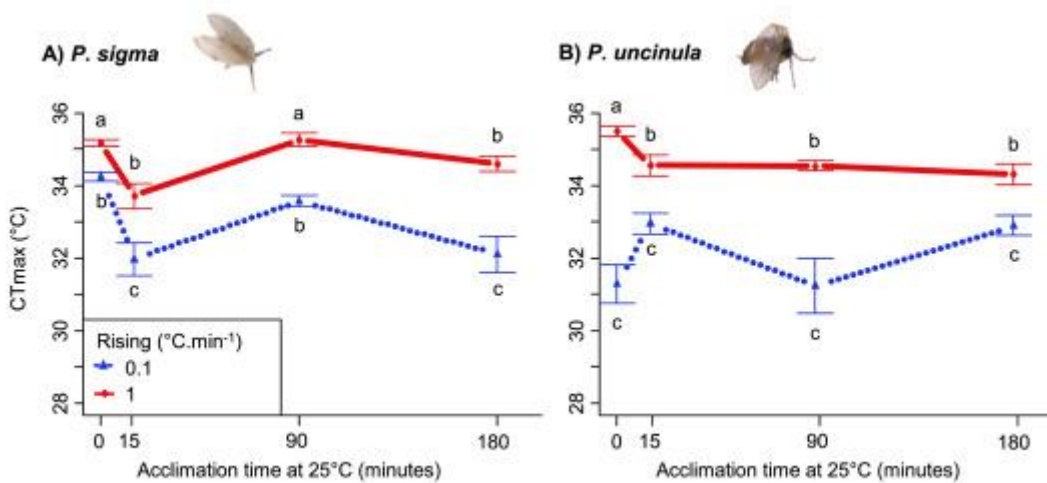
587

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.