



**HAL**  
open science

# Hot and cold waves decrease sperm production and bias sex ratio in the parasitoid wasp *Cotesia typhae* (Hymenoptera, Braconidae)

Christophe Bressac, Ahmed El-Sabrou, Fatma Kifouche, Melissa Anne, Florence Mougel, Claire Capdevielle-Dulac, Laure Kaiser

## ► To cite this version:

Christophe Bressac, Ahmed El-Sabrou, Fatma Kifouche, Melissa Anne, Florence Mougel, et al.. Hot and cold waves decrease sperm production and bias sex ratio in the parasitoid wasp *Cotesia typhae* (Hymenoptera, Braconidae). *Journal of Insect Physiology*, 2023, Environmental stress physiology and toxicology: necessary insights in a changing world, 149, pp.104553. 10.1016/j.jinsphys.2023.104553 . hal-04225245

HAL Id: hal-04225245

<https://hal.science/hal-04225245v1>

Submitted on 2 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - ShareAlike 4.0 International License

# 1 Hot and cold waves decrease sperm production and bias sex ratio in 2 the parasitoid wasp *Cotesia typhae* (Hymenoptera, Braconidae)

3

4 Christophe Bressac<sup>1</sup>, Ahmed El Sabrout<sup>1,2</sup>, Fatma Kifouche<sup>1</sup>, Melissa Anne<sup>3</sup>, Claire Capdevielle-Dulac<sup>3</sup>,  
5 Florence Mougel<sup>3</sup>, Laure Kaiser<sup>3</sup>

6 <sup>1</sup> IRBI, UMR 7261 CNRS, Institut de Recherche sur la Biologie de l'Insecte, Faculté des Sciences et  
7 Techniques, Université de Tours, Tours, France

8

9 <sup>2</sup> Applied Entomology and Zoology Department, Faculty of Agriculture (El-Shatby), Alexandria  
10 University, 21526 Alexandria, Egypt

11

12 <sup>3</sup> EGCE, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie,  
13 91190 Gif-sur-Yvette, France.

14

15 \*Author for Correspondence: christophe.bressac@univ-tours.fr

16

## 17 Abstract

18 Parasitoid wasps are haplodiploid, meaning that sperm stored by egg laying females are only used to  
19 produce daughters. Thus, the sex ratio of the offspring depends on the availability of sperm after  
20 mating. In these insects, males are sensitive to temperature at the pupal stage. This stress leads to  
21 subfertility due to a drastic reduction in the number of sperm produced and transferred to females.  
22 Experiments were conducted under controlled conditions on the parasitoid wasp *Cotesia typhae*  
23 (Hymenoptera, Braconidae), a natural enemy of the invading pest *Sesamia nonagrioides*  
24 (Lepidoptera, Noctuidae). At 25-27°C, sperm production was measured for 7 days, and found to  
25 reach a plateau at the third day of adult life. It leads to a final amount around 25,000 sperm per  
26 male. A male can successfully inseminate at least 10 females, producing predominantly female  
27 offspring. Sperm production decreased significantly after 1 day of pupal exposure to heat at 34 or  
28 36°C and 7 days of cold at 0, 5 or 10°C. This highlights that both cold and heat are stressful. After  
29 mating with one male treated at 10 or 34°C, females store fewer sperm than the control, and  
30 produce fewer daughters. The sex ratio of the offspring is male biased when males experienced  
31 temperature stresses during development, like other parasitoid wasps. In the field, *C. typhae*  
32 populations would be affected by heat and cold, at least at the pupal stage. This lowers  
33 overwintering risk in case this biological agent was introduced in Europe. This risk is both economical,  
34 as companies seek to establish costly continuous production to sell beneficial insects, and ecological  
35 as the introduced population would not settle in the ecosystem. Lastly, the transport and storage of  
36 this insect of agronomic interest would need to consider temperature variations to ensure successful  
37 application.

## 39 Key words

40 Reproduction; temperature stress; male fertility; overwintering; biocontrol

## 41 Introduction

42 Parasitoid wasps are extensively studied for their ability to reduce insect pests in cultures and  
43 storage, but also for their reproductive system. In these insects, sex determination is based on  
44 haplodiploidy, whereby males are haploid and females, diploid. As only female offspring require the  
45 use of sperm at oviposition, sex ratio is determined by the amount of sperm available to egg-laying  
46 females (Quicke, 1997, Chevrier and Bressac, 2002). Virgin females only lay male offspring, which are  
47 unable to control pests, and compromise biological control programmes. Therefore, sex ratio plays a  
48 major role for the success of parasitoid wasps in pest management. In most species, the sex ratio is  
49 female biased (Quicke, 1997), and whereas females usually mate only once (monoandry), males can  
50 mate multiple times (polygyny). The sex ratio results from an evolutionary trade-off, as an excess of  
51 males reduces egg laying and the control of targeted pests, and as a deficit in males results in  
52 unmated or badly inseminated females that lay male-biased clutches, compromising the efficiency of  
53 parasitism (Bressac et al., 2009; Chevrier et al., 2019). However, even when the proportion of males  
54 appears optimal, a decreased quality of reproduction may occur due to the suboptimal fertility of  
55 individual males. In many parasitoid wasps, so-called subfertile males produce a reduced amount of  
56 sperm (Lacoume et al., 2007; Chevrier et al., 2019; Chirault et al., 2015; El Sabrout et al., 2021).

57 Insects are vulnerable to temperature variations because of their small size and ectothermic  
58 physiology (Ma et al., 2021). Heat waves at the pupal stage can reduce sperm production and thus  
59 induce sex ratio biases in the following generations. This thermal sensitivity was found in diverse  
60 parasitoid wasps belonging to the families Pteromalidae (*Anisopteromalus calandrae* –Nguyen et al.,  
61 2003; *Dinarmus basalis* – Lacoume et al., 2007; *Nasonia vitripennis* – Chirault et al., 2015), and  
62 Braconidae (*Microplitis rufiventris* – El Sabrout et al., 2021).

63 In the field, insects experience temperature variations that go beyond standard ambient  
64 temperature variation and they use many strategies to control the temperature actually felt, such as  
65 individual behaviour (Ma et al., 2021) or specific morphology (Bota-Sierra et al., 2022). However,  
66 most of these adaptations are not effective for immobile developmental stages, as are the pupae of  
67 holometabolous insects.

68 Males are generally more sensitive to heat than females (Chen et al., 2018), and the pupa is a critical  
69 stage because this is when spermatogenesis is most intensive (Chirault et al., 2016). Most insect  
70 males survive a limited heat shock and may not become sterile. However, single heat periods induce  
71 subfertility, as most males reproduce with lower performance than control males (Chevrier et al., 2019).

72 Moreover, in some parasitoid wasps, cold temperatures associated with short photoperiod induced a  
73 reproductive diapause of females (Chen et al., 2012). However, there is a lack of empirical evidence  
74 of the effect of lower temperatures on males.

75 *Cotesia typhae* (Hymenoptera, Braconidae) is a promising insect for the biocontrol of the maize pest  
76 *Sesamia nonagrioides* (Lepidoptera, Noctuidae) in Europe. *C. typhae* has been described as a  
77 specialized clade of *Cotesia sesamiae* in eastern Africa where it develops exclusively on *S.*  
78 *nonagrioides* on two associated plants (Kaiser et al., 2017; Gauthier et al 2018). In its native range, *C.*  
79 *typhae* is exposed to average temperatures between 20°C and 30°C with extremes varying between  
80 15°C and 35°C (West Kenya, Kobodo, NASA Langley Research Center (LaRC),  
81 <http://power.larc.nasa.gov>). *C. typhae* is a gregarious species, and females lay between 80 and 100  
82 eggs in their host (Benoist et al., 2020). After endoparasitic ovo-larval development, parasitoid larvae  
83 emerge from the host and spin their cocoon. The life cycle lasts 3 weeks at 27°C and adults live a few  
84 days in these laboratory conditions. The thermal resistance (CT min and CT max) of this insect  
85 remains to be investigated in the perspective of its introduction in Europe, where we expect heat  
86 shocks in summer amplified by climate change, and cold periods during winter.

87 We conducted laboratory experiments to characterize male fertility and to simulate summer and  
88 winter thermal variations on males at the pupal stage. The aim was to study a possible sensitivity to  
89 heat waves that are increasing in frequency due to climate change, and winter survival. This thermal  
90 sensitivity is also of major importance for storage and transport, in the perspective of production and  
91 commercialisation of *C. typhae* as a biological control agent. We focused on the main components of  
92 male fertility: sperm production by males, post-copulatory storage by females, and offspring sex  
93 ratio.

94

## 95 [Material and methods](#)

### 96 [Insect production](#)

97 The laboratory strain of the host *S. nonagrioides* originates from different localities of Southwest  
98 France and is refreshed yearly by adding field collected larvae. Eggs collected in the rearing cages  
99 were placed at 21°C for 9 days. After gentle wash with chlorine-based disinfectant (Daquin), rinse  
100 and dry, they were transferred into a baby bottle filled with artificial diet (adapted from Overholt et  
101 al., 1994). Bottles were placed in a rearing incubator at 26°C with 60-70 % RH and 16:8 LD cycle, until  
102 pupae formation to get adults for the rearing (5 weeks later), or until the formation of five-instar  
103 larvae (3-week-old) for parasitism.

104 The Wasp colony was initiated from individuals emerging from parasitized larvae collected in the  
105 field near Kobodo in Kenya (0.679S, 34.412E; West Kenya) in 2019. Experimental breeding of *C.*

106 *typhae* was performed in a containment laboratory at the Evolution, Génome, Comportement et  
107 Ecologie (EGCE, Gif-sur-Yvette, France) since 2015. Parasitized host larvae are placed in Petri dishes  
108 with artificial diet and kept at 27°C, 60-70% RH and 16:8 light-dark (LD) cycle in rearing incubators.  
109 After 10 days, host larvae are retrieved from the artificial diet and placed in folded disposal  
110 absorbent paper to provide a dry environment. The 3<sup>rd</sup> larval instars egress from the host larva 2 days  
111 later to pupate in silk cocoons, giving a larval development of 12 days. Cocoon masses are then  
112 collected and placed in a 15L bucket with access holes closed by foam plugs (cage) at 27 °C and 12h  
113 LD cycle until adult emergence (5 or 6 days). Honey droplets are deposited on the cage walls, and a  
114 paper ball soaked with water is placed in the centre of the cage, to provide food and water for the  
115 emerging adults.

116

### 117 Sperm in seminal vesicles and age

118 Experiments occurred under controlled conditions, at a temperature between 25 and 27°C. Host  
119 larvae were parasitized by virgin *C. typhae* females to obtain only male offspring. After egress from  
120 the host, parasitic larvae weave a silk cocoon and transform into pupae. Each day, some cocoons  
121 were opened to follow pupal development, and determine the day of emergence. After emergence,  
122 adult males were transferred to petri dishes by groups of 10 with a cotton ball saturated with water  
123 and a drop of honey as nutritional resources. They were maintained at 25-27°C with a 12h-12h light-  
124 dark cycle.

125 Males were collected at the age of -1 day (one day before emergence, n=25), 0 day (the day of  
126 emergence, n=30), 1 day (n=30), then 3 (n=17), 5 (n=19), and 7 days (n=6), and their reproductive  
127 apparatus isolated with fine forceps in a drop of saline solution. Following methodology of El Sabrout  
128 et al. (2021), seminal vesicles were isolated in saline solution on a microscope glass slide, their  
129 content spread out, then fixed in 4% paraformaldehyde, dried, post fixed with 99% ethanol, dried  
130 again, and labelled with DAPI. After rinsing with tap water, slides were mounted in Immu-Mount  
131 (Thermo Inc.) and observed under a fluorescence microscope with a UV filter and a x100 objective  
132 under immersion. All sperm on the slide were counted to avoid any bias due to dilution, which was  
133 impossible because in very short sperm insects sperm would lose their integrity when diluted (El  
134 Sabrout et al., 2021).

### 135 Male multiple mating

136 To perform controlled mating virgin males and females were obtained from individual cocoons  
137 isolated from cocoon masses and kept in tubes with a moistened cotton wool ball and a drop of  
138 honey at 25-27°C until the emergence. Ten males were separately placed with successive females  
139 until each male had mated with ten different females. The ten matings per male were performed  
140 over two days with 1–2-day-old males. The mated females were allowed to oviposit in *S.*

141 *nonagrioides* caterpillars one or two days later, and the number of male and female offspring were  
142 counted after the development of the next *C. typhae* generation to determine sex ratio calculated as  
143 females divided by the total offspring.

#### 144 Cold and hot treatments

145 Heat treatments were applied on 960 male pupae inside their cocoons. They were from 16 host  
146 larvae parasitized by virgin *C. typhae* females for three successive repetitions, all from the same  
147 laboratory colony. Cocoons were collected 1 day after egress from the host - the pupa is white with  
148 red eyes - and transferred in petri dishes with a moistened cotton wool ball and a drop of honey.  
149 They were maintained for 24h in climatic chambers at 34, 36°C or 38°C with 60% relative humidity,  
150 then returned to 25-27°C until complete development and emergence. This protocol was applied in  
151 other parasitoid wasps (Chirault et al., 2015; El Sabrout et al., 2021). One set of cocoons per  
152 repetition was maintained at 25-27°C as a control.

153 Three repetitions of cold treatments were applied on 1980 male pupae inside their cocoons. They  
154 were from 28 host larvae parasitized by virgin *C. typhae* females. The protocol was the same as for  
155 heat treatments, but two durations have been tested, 24 hours and 7 days (168h). Preliminary  
156 experiments show that pupae do not survive after 2 hours at -5°C; therefore, only zero and positive  
157 temperatures were tested. Cold temperatures were 0°C, 5°C and 10°C. After the cold treatment,  
158 cocoons were returned to 25-27°C until emergence.

#### 159 Sperm stored by females and egg laying

160 One virgin female was introduced in a petri dish with one virgin male and continuously observed until  
161 copulation. Then the females were isolated for at least 3 hours in a petri dish with a cotton ball  
162 saturated with water and either tested for egg laying or dissected for sperm count. All males were 0  
163 day old (ie tested the day of their emergence) to avoid any bias due to ageing. The cold treatment  
164 was 7 days at the temperature of 10°C, chosen because it is representative of a mild winter in  
165 Europe, n=7 control females mated with experimental males were tested for sperm in spermathecae,  
166 n=11 females for egg-laying. At 25-27°C (for 1 day, control), n=35 control females mated with control  
167 males were tested for sperm in spermathecae, n=35 for egg-laying. For the 1-day hot treatment, 34°C  
168 was chosen because 36°C induced mortality (40%), and n=27 control females mated with heat  
169 treated males were tested for sperm in spermathecae, n=6 for egg-laying. The number of  
170 experimental males after a cold treatment is low because the cold stay extended the pupal period,  
171 which made it difficult to synchronise males with the availability of virgin young females to mate with  
172 them the day of their emergence. For 34°C, only 6 males were tested for their offspring after  
173 copulation because this test was conducted after showing that sperm numbers were dramatically  
174 decreased by a heat shock.

175 Spermathecae were considered for their sperm content. Sperm labelling by DAPI and counting  
176 methods were as for males. Ten spermathecae were photographed under a x100 objective to  
177 measure their size. Volume was calculated from length and width of this bulb-shaped organ.

178 Females that mated once with either a cold-treated (10°C), control or heat-treated (34°C) male were  
179 offered two hosts at 1–2-day interval for oviposition. Stinging behaviour was observed to prevent  
180 multiple parasitism. Stung hosts were isolated in a petri dish with food in the climate chamber until  
181 either the egress of *C. typhae* cocoons, host pupation (i.e. parasitism failed), or host death. When  
182 offspring emerged from their cocoons, females and males were counted to calculate the sex-ratio.

### 183 [Statistics](#)

184 All statistics and graphs were performed with R studio (version 1.3.1093, RStudio Team, 2020). One-  
185 or two-way ANOVA followed by Tukey tests were applied to sperm counts of control and treated  
186 males when data conformed to ANOVA model assumptions (equal variance among conditions and  
187 normal distribution of residuals checked on diagnostic plots). Otherwise, non-parametric tests  
188 (Kruskall Wallis followed by pairwise Mann-Whitney tests) were performed. For each dataset  
189 (seminal vesicle or spermatheca content), factors tested were (1) temperature treatment and (2) age  
190 when relevant.

191 Sex ratio of offspring of the ten successive females mated with one male was analysed as a binomial  
192 variable and compared between mating ranks - encoded as a factor due to the lack of a discernible  
193 trend - using a mixed model (glmer) and male identity as a random effect. This model was compared  
194 to a simplest one that only take into account the male's identity using a Chisq-test. Spermathecae  
195 were measured with Image J (version 1.54, NIH, USA). Effective fertilization of mated females was  
196 first compared between control and temperature-treated males by comparing the ratio of clutches  
197 with females obtained in the different treatments. Chisq-test were applied, or Fisher exact tests  
198 when at least one cell of the table of expected values was below 5. Then, sex ratios of mixed clutches  
199 (comprising both males and females) were compared by Kruskal-Wallis tests followed by pairwise  
200 Student or Mann-Whitney tests, according to the conformity of the data to parametric tests.  
201 Offspring output of all progenies (mixed or male-only) were compared by ANOVA followed by Tukey  
202 tests. Means are presented  $\pm$  SD in the text.

### 203 [Results](#)

#### 204 [Sperm in seminal vesicles and age](#)

205 Sperm increased in seminal vesicles of control males over several days (ANOVA,  $F_{5-122}=200.1$ ,  
206  $p<0.001$ , Figure 1). After the 3<sup>rd</sup> day, sperm do not longer increase significantly. Sperm were present  
207 in seminal vesicles before emergence, and their maximum was over 20,000 after 3 days. At 7 days,  
208 counts were from surviving males since many of them died before. After 5 days in standard

209 conditions, 50% were alive and no male survived after 9 days. In the following experiments, 3 days  
210 will be considered as the maximum sperm stock for males.

211

### 212 Male multiple mating

213 Ten males were each mated with ten different females successively for a total number of 100  
214 females, and between 5 and 10 cocoon clutches were finally obtained for each male, corresponding  
215 to between 6 and 9 clutches for each mating rank. Six progenies contained no females, which could  
216 be due to a failure in sperm transfer during copulation and were discarded from statistical analysis.  
217 There was a significant effect of mating rank on the offspring sex ratio (Chisq=68.9, df=9, p-value  
218 <0.0001) with no evidence of sperm depletion. Based on the comparison of fixed effect estimates  
219 with rank one as a reference, the sex ratio was significantly higher in rank 2, lower in ranks 3 and 4,  
220 and not significantly different in ranks 5 to 10.

221

### 222 Cold and hot treatments

223 Treatment at 38°C for the pupae was not considered because it resulted in the death of all males,  
224 and 36°C only led to 60% emergence. In all other temperature treatments, all males emerged and  
225 were alive at 3 days. For cold treatments, durations of 24h at either 0, 5 and 10°C induced no effect  
226 on sperm production in comparison with control males at both ages (ANOVA,  $F_{3-147}=1.46$ ,  $p=0.23$ ).  
227 Therefore, only 7-day durations were considered for further testing of cold temperatures.

228 For sperm counted in the seminal vesicles of virgin males after heat treatment at the pupal stage, all  
229 temperatures and all ages were significantly different (2-way ANOVA, Temperature  $F_{6,293}=62.3$ ,  
230  $p<0.001$ ; Age  $F_{2,293}=180.4$ ,  $p<0.001$ ; Temperature + Age  $F_{12,293}=39.6$ ,  $p<0.001$ , figure 3). For all males,  
231 whatever the temperature treatment, sperm increased from one day before emergence to 3 days  
232 post emergence. Sperm productions in 25°C and 28°C treatments showed similar profiles, and were  
233 not significantly different (2-way ANOVA, Temperature + Age  $F_{2,78}=0.65$ ,  $p=0.42$ ). Exposure to cold for  
234 7 days or heat for one day decreased sperm production. For cold treatments, age had an effect on  
235 sperm counts, but no difference could be observed between temperatures and the interaction  
236 between temperature and age is not significant (2 way ANOVA, temperature  $F_{2,127}=0.23$ ,  $p=0.63$ ; age  
237  $F_{2,127}=24.5$ ,  $p<0.001$ ; Temperature + Age  $F_{4,127}=0.002$ ,  $p=0.97$ ). However, for heat, both temperature  
238 and age have an effect on sperm, and the interaction between temperature and age is significant;  
239 34°C had less effect than 36°C (2 way ANOVA, Temperature  $F_{1,90}=20.71$ ,  $p<0.001$ ; Age  $F_{2,90}=136.68$ ,  
240  $p<0.001$ ; Temperature + Age  $F_{2,90}=11.85$ ,  $p<0.001$ ).

### 241 Mating

242 Sperm stored in spermathecae



243 Male were able to mate with females, whatever their temperature treatment at the pupal stage.  
244 Copulation times were not recorded (lasting some seconds), but no obvious difference was observed  
245 between males, except that few (not counted) treated at 34°C needed a longer time mounting or  
246 detaching from females. The reservoir of the spermatheca is a minute chitinized capsule, measuring  
247 26.5 x 15.6  $\mu\text{m}$ ; its volume is 306  $\mu\text{m}^3$ , that is 3.06 E-4 nl (figure 4a). Sperm stored in spermathecae  
248 differed between heat treatment of males (Figure 4b, ANOVA,  $F_{2,70}=19.10$ ,  $p<0.001$ ). Mean sperm  
249 numbers stored by females after male treatment at either 10, 25 or 34°C were respectively 83 $\pm$ 15.1,  
250 235 $\pm$ 22.0, 61 $\pm$ 18.0 (numbers on figure 4b).

#### 251 Egg-laying

252 Parasitic success can be defined on several level for any standard females mated with control or  
253 experimental males, while failures could be due to host resistance or death. The temperature  
254 treatment of males did not affect the development (and resistance) of the hosts or their death. A  
255 measure of parasitic success is the presence of parasitoid cocoons, from which males or females  
256 emerge. Male treatment did not impact overall offspring production (Figure 5, P1, ANOVA  $F_{2,49}=0.9$ ,  
257  $p=0.4$ ; P2, ANOVA  $F_{2,49}=1.05$ ,  $p=0.36$ ). However, the reproductive success of males (father) is only  
258 reflected in the number female offspring, in haplodiploids. The number of unfertilized progeny, i.e.  
259 without females, increased when males faced with either cold or hot treatment during pupal  
260 development, but this change is not significant (P1,  $\text{Chisq}=3.3$ ,  $\text{df}=2$ ,  $p=0.19$ ,  $p=0.28$ ; P2, Fisher exact  
261 test,  $p=0.48$ ; hereafter P1 and P2 refer to the first and second hosts in which a female oviposited).  
262 However, the sex ratio of mixed progenies (containing females and thus reflecting the sperm use)  
263 differed between male treatments for both first and second egg-laying (Figure 6, P1, Kruskal Wallis  
264  $\text{Chisq}=30.2$ ,  $\text{df}=2$ ,  $p<0.001$ ; P2, Kruskal Wallis  $\text{Chisq}=19.2$ ,  $\text{df}=2$ ,  $p<0.001$ ). Of note only two out of five  
265 clutches contained females for 34°C treated males at P2. Adding the outputs resulting from the first  
266 and second mating gave the overall production of female offspring, hence the minimum number of  
267 sperm actually used by each female. The mean values of sperm used per female are respectively 58  
268 for 10°C stressed males, 78 for 25-27°C control males and 12 for 34°C stressed males, when  
269 considering only offspring with at least one female (Figure 6).

270

## 271 Discussion

272 One of the challenges of establishing biological agents concerns their adaptability to new  
273 environmental conditions, including temperature variations. Here we found that males of the  
274 parasitoid wasp *Cotesia typhae* are strongly affected by heat or cold waves in the early pupal stage.  
275 Sperm production is reduced when pupae have been exposed to cold for 7 days or heat for one day.  
276 As a consequence post-copulatory sperm storage by females appeared reduced, and the resulting sex  
277 ratio of the offspring is strongly male biased, especially when males were exposed to heat. This  
278 sensitivity could compromise both winter and summer resistance of *C. typhae* in maize fields for  
279 controlling its host *S. nonagrioides*.

280 The physiological response of insects to heat is well documented, especially for pests under  
281 controlled conditions (Neven, 2000, Kirk Green et al., 2019). Generally, heat sensitivity was  
282 investigated through an ecophysiology approach (Gonzalez-Tokman et al., 2020; Harvey et al., 2020).  
283 It was found to result in an metabolic increase monopolising energy for individual survival to the  
284 detriment of other non-essential functions, such as reproduction, and in the overproduction of toxic  
285 metabolites, such as amines inducing oxidative stress. This led to the proposal of “thermal fertility  
286 limits” which is the highest temperature where reproduction is physiologically possible (Walsh et al.,  
287 2019), such limit being not restricted to insects. Unfortunately, this is poorly described for males,  
288 where consequences of heat seem to be variable. For instance, males of the moth *Plutella xylostella*  
289 are negatively affected by a short heat period, but apparently less than females (Zhang et al., 2013),  
290 however, sperm production was not considered in this experimental study. In *Drosophila suzukii*,  
291 males and females show a severe reduction of their reproduction after a heat stress, and males are  
292 more sensitive than females (Kirk Green et al., 2019). In this dipteran pest, sperm production was not  
293 actually quantified but the reduction of sperm was assessed by a whole examination of testes and  
294 seminal vesicles after nuclei staining.

295 A decrease of sperm production in parasitoid wasps after a heat period during pupal stage is well  
296 known for both ecto- and endo-parasitoids (Nguyen et al., 2013, Chirault et al., 2015, El-Sabrout et  
297 al., 2021) but the consequences of cold are less documented. Lacoume et al. (2007) showed that  
298 after a severe cold shock (-18°C during 1h) at the pupal stage, males of the Pteromalid *Dinarmus*  
299 *basalis* had 50% less sperm than controls but females mated to cold-shocked males produced as  
300 many daughters as those mated to control males. The only fitness reduction was the number of  
301 females inseminated by a male, which was 15 for controls and 11 for cold shocked males. In *C.*  
302 *typhae*, control males were able to inseminate at least 10 females with no apparent reduction in the  
303 production of daughters. Present results show that the sex ratio is mainly female biased, with on  
304 average 80% females, but 5% were not inseminated and 10% produced a balanced or male-biased  
305 sex ratio. In the closely related species *Cotesia congregata*, one male can inseminate up to 15  
306 females, and the sex ratio gradually decreases along the rank of females (Bredlau et al., 2020).

307 Although heat- or cold-stressed males were not tested for successive mating here, the ability of  
308 males to inseminate multiple females should be reduced by temperature at the pupal stage, leading  
309 to females that are either virgin or sperm-constrained and cannot produce offspring with an optimal  
310 sex ratio.

311 Cold treatment experimented here could not be considered as a cold shock, because temperatures  
312 are null or positive, and the duration is much longer than a shock. Thus, physiological effects of cold  
313 could be compared to a chill torpor that is experienced in the field during overwintering. In *C. typhae*  
314 males, a 7day period at either 0, 5 or 10 °C did not induce diapause, because the development is  
315 interrupted and restarts as the temperature rises. This is a transitory state of dormancy at the  
316 immature stage of pupae. In insects, stage resisting to cold can differ between species (Dansk, 2002),  
317 as an example mosquito eggs tolerate cold periods better than other stages (Diniz et al., 2017). In  
318 parasitoid wasps, the induction of dormancy is very variable, triggered by the host, climatic  
319 conditions, light rhythm, and all parameters together (Polgar et al., 1995). Previous results show  
320 parasitoids may survive at the larval stage within the host body, as for three parasitoids species of  
321 the invading pest *Rhagoletis completa* (Tephritidae) (Rull et al., 2019). The resistance of *C. typhae*  
322 other stages as adults, eggs and larvae when they are inside the host, would require further  
323 investigations.

324 Heat and cold treatments of male pupae led to a decrease in sperm production in *C. typhae* males. A  
325 temperature dependant heat stress was showed, this decrease being higher after 36°C than 34°C.  
326 This phenomenon was also observed in other tested experimentally parasitoids (Nguyen et al., 2013,  
327 Chirault et al., 2015, El-Sabroun et al., 2021). In contrast, sperm production does not differ in males  
328 between a 0, 5 or 10°C exposure for 7 days, after which it is equally severely diminished. The  
329 physiological stress induced by cold does not appear to be temperature dependant; this suggests a  
330 physiological mechanism that is specifically triggered by lower temperatures and with the same  
331 intensity when it drops below a threshold. This do not preclude that an intermediate effect on males,  
332 as evidenced for heat, could be induced by less rigorous conditions that are encountered in  
333 temperate areas. Whereas physiological changes induced by heat in insects are well described  
334 (Gonzalez-Tokman et al., 2020; Kirk Green et al., 2019), cold stress induction is less documented,  
335 especially in relation to male reproduction.

336 The production of the second offspring is less than half that of the first. It shows that oocytes are  
337 limited, confirming that *C. typhae* females are strictly pro-ovigenic (Jervis et al., 2001), with ovaries  
338 producing oocytes in only one wave during preimaginal stages. Independently of the male  
339 treatments, the sex ratio does not change between 1<sup>st</sup> to 2<sup>nd</sup> egg laying, evidencing that females are  
340 not able to allocate sperm in the first egg laying even if the low availability of this reproductive  
341 resource results in a non-optimal progeny. This does not preclude subsequent mating from being less  
342 efficient, because in most parasitoid wasps, females mate only once and subfertile or sterile males

343 induce pseudo-virgin -females that receive fewer or even no sperm and lay only males (Damiens and  
344 Boivin, 2005).

345 Sperm are produced in larger amount than needed for storage by females and production of female  
346 offspring. The spermatheca is small, as in the related species *C. congregata* (Bredlau et al., 2020), and  
347 contains fewer spermatozoa, ie 250 vs 1000 in *C. congregata*. Regarding sperm efficiency, females  
348 mated with control males lay on average 80 daughters, which is under the average 250 sperm stored  
349 in the spermathecae and represent 32% of sperm being used. In females mated with cold or heat  
350 sperm-decreased males, this ratio is respectively of 72% for 10°C treated males, and 20% for 34°C  
351 treated males (with a very reduced number of males tested). Such relatively high sperm efficiency  
352 was found in other parasitoid wasps (Bredlau et al., 2020; Chirault et al., 2015), evidencing that  
353 sperm stored by females is of optimal quality (Damiens et al., 2002).

354 As a summary, cold and heat exposure of the pupal stage of *C. typhae* have negative effects on the  
355 production of sperm by males, this decreases sperm storage in females after mating, and results in  
356 constrained females that lay an offspring containing fewer females than the controls. Finally, *C.*  
357 *typhae* males are highly sensitive to temperature variations that could be encountered in Europe.  
358 The resulting stress is visible on sperm production and transfer. In practice, overwintering as cocoons  
359 is unlikely, and will result in subfertile males and subsequent strongly male biased sex ratios.  
360 Refrigerated transport of pupae is also not recommended.

361

## 362 [Acknowledgements](#)

363 The authors thank Alain Peyhorgues for providing field collected *S. nonagrioides* individuals for the  
364 laboratory rearing at EGCE; Remi Jeannette and Hubert Marteau for *S. nonagrioides* rearing; Paul-  
365 Andre Calatayud and the *Cotesia* rearing team at *ICIPE* (Nairobi, Kenya) -Julius Obonyo, Josphat  
366 Akhobe and Enock Mwangangi - for insect collection and shipment to refresh EGCE rearing, as well as  
367 Elisabeth Herniou for discussions. Work on Kenyan insects was done under the juridical framework of  
368 material transfer agreement CNRS 072057/IRD 302227/00. This study was co-funded by the French  
369 National Research Agency (ANR) and the National Agency for Biodiversity (AFB) (grant CoteBio  
370 ANR17-CE32-0015-02 to L.K.) and by the BASC consortium of Université Paris Saclay. Two anonymous  
371 reviewers gave helpful suggestions, especially on statistical analyses. Elisabeth Herniou gave helpful  
372 comments on the revised manuscript and corrected the English.

373

374 [References](#)

- 375 Benoist, R., Capdevielle-Dulac, C., Jeannette, R., Calatayud, P.A., Drezen, J.M., Dupas, S., Le Rouzic, A.,  
376 Le Ru, A., Moreau, L., Vanb Dijk, E., Kaiser, L., Mougel, F., 2020. Quantitative trait loci involved in the  
377 reproductive success of a parasitoid wasp. *Molecular Ecology*, 29, 3476-3493.  
378 <https://doi.org/10.1111/mec.15567>
- 379 Bota-Sierra, C.A., García-Robledo, C., Escobar, F., Novelo-Gutiérrez, R., Londoño, G.A., 2022.  
380 Environment, taxonomy and morphology constrain insect thermal physiology along tropical  
381 mountains. *Functional Ecology*, 36, 1924–1935. <https://doi.org/10.1111/1365-2435.14083>
- 382 Bredlau, J.P., El-Sabrou, A.M., Bressac, C., 2020. Reproductive context of extremely short sperm in  
383 the parasitic wasp *Cotesia congregata* (Hymenoptera: Braconidae). *Biological Journal of the Linnean*  
384 *Society*, 131, 384–395. [10.1093/biolinnean/blaa111](https://doi.org/10.1093/biolinnean/blaa111).
- 385 Bressac, C., Do Thi Khanh, H., Chevrier, C., 2009. Effects of age and repeated mating on male sperm  
386 supply and paternity in a parasitoid wasp. *Entomologia Experimentalis et Applicata*, 130, 207-213.
- 387 Chen, H., Zhang, H., Zhu, K.Y., Throne, J.E., 2012. Induction of reproductive diapause in *Habrobracon*  
388 *hebetor* (Hymenoptera: Braconidae) when reared at different photoperiods at low temperatures.  
389 *Environmental Entomology*. 41, 697-705. doi: 10.1603/EN11311
- 390 Chen, H., Zheng, X., Luo, M., Guo, J., Solangi, G.S., Wan, F., Zhou, Z., 2018. Effect of short-term high-  
391 temperature exposure on the life history parameters of *Ophraella communa*. *Scientific Reports*, 18,  
392 13969. doi: 10.1038/s41598-018-32262-z.
- 393 Chevrier, C., Bressac, C., 2002. Sperm storage and use after multiple mating in *Dinarmus basalis*  
394 (Hymenoptera: Pteromalidae). *Journal of Insect Behavior*, 15, 385–398.
- 395 Chevrier, C., Nguyen, T.M., Bressac, C., 2019. Heat shock sensitivity of adult male fertility in the  
396 parasitoid wasp *Anisopteromalus calandrae* (Hymenoptera, Pteromalidae). *Journal of Thermal*  
397 *Biology*, 85, 102419, doi:10.1016/j.jtherbio.2019.102419.
- 398 Chirault, M., Lucas, C., Goubault, M., Chevrier, C., Bressac, C., Lécureuil, C., 2015. A combined  
399 approach to heat stress effect on male fertility in *Nasonia vitripennis*: from the physiological  
400 consequences on spermatogenesis to the reproductive adjustment of females mated with stressed  
401 males. *PLoS ONE* 10, e0120656. [10.1371/journal.pone.0120656](https://doi.org/10.1371/journal.pone.0120656).
- 402 Chirault, M., van de Zande, L., Hidalgo, K., Chevrier, C., Bressac, C., Lécureuil, C., 2016. The spatio-  
403 temporal partitioning of sperm by males of the prospermatogenic parasitoid *Nasonia vitripennis* is in  
404 line with its gregarious lifestyle. *Journal of Insect Physiology*, 91-92, 10-17.

405 Damiens, D., Boivin, G., 2005. Male reproductive strategy in *Trichogramma evanescens*: Sperm  
406 production and allocation to females. *Physiological Entomology*, 30, 241–247. doi:10.1111/j.1365-  
407 3032.2005.00453.x.

408 Damiens, D., Bressac, C., Brillard, J.P., Chevrier, C., 2002. Qualitative aspects of sperm stock in males  
409 and females from *Eupelmus orientalis* and *Dinarmus basalis* (Hymenoptera: Chalcidoidea) as revealed  
410 by dual fluorescence. *Physiological Entomology*, 27, 97-102.

411 Danks, H.V., 2002. The range of insect dormancy responses. *European Journal of Entomology*, 99,  
412 127-142. doi:10.14411/eje.2002.021.

413 Diniz, D.F.A., de Albuquerque, C.M.R., Oliva, L.O., Varjal de Melo Santos, M.A., Junqueira Ayres C.F.,  
414 2017. Diapause and quiescence: dormancy mechanisms that contribute to the geographical  
415 expansion of mosquitoes and their evolutionary success. *Parasites Vectors*, 10, 310.  
416 <https://doi.org/10.1186/s13071-017-2235-0>.

417 El-Sabrou, A.M., Hegazi, E., Khafagi, W., Bressac, C., 2021. Sperm production is reduced after a  
418 heatwave at the pupal stage in the males of the parasitoid wasp *Microplitis rufiventris* Kok.  
419 (Hymenoptera; Braconidae). *Insects*, 12, 862. 10.3390/insects12100862.

420 Gauthier, J., Gayral, P., Le Ru, B.P., Jancek, S., Dupas, S., Kaiser, L., Gyapay, G., Herniou EA., 2018.  
421 Genetic footprints of adaptive divergence in the bracovirus of *Cotesia sesamiae* identified by  
422 targeted resequencing. *Molecular Ecology*, 27, 2109-2123. <https://doi.org/10.1111/mec.14574>

423 González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A.,  
424 Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and ecological  
425 implications in a warming world. *Biological Reviews*, 3, 10. 1111/brv.12588

426 Harvey, J.A., Heinen, R., Gols, R., Thakur, M.P., 2020. Climate change-mediated temperature  
427 extremes and insects: from outbreaks to breakdowns. *Global Change Biology*. 2020 26, 6685-6701.  
428 doi: 10.1111/gcb.15377.

429 Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A. Kidd, N.A.C., 2001. Life-history strategies in  
430 parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology*, 70, 442-458.  
431 <https://doi.org/10.1046/j.1365-2656.2001.00507.x>.

432 Kaiser, L., Fernandez-Triana, J., Capdevielle Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R.,  
433 Calatayud, P-A., Dupas, S., Herniou, E.A., Jeannette, R., Obonyo, J., Silvain, J-F., Le Rü, B., 2017.  
434 Systematics and biology of *Cotesia typhae* sp. n. (Hymenoptera, Braconidae, Microgastrinae), a  
435 potential biological control agent against the noctuid Mediterranean corn borer, *Sesamia*  
436 *nonagrioides*. *Zookeys*, 682, 105-136. ISSN 1313-2989.

437 Kirk Green, C., Moore, P.J., Sial, A.A., 2019. Impact of heat stress on development and fertility of  
438 *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Journal of Insect Physiology, 114, 45-52.  
439 <https://doi.org/10.1016/j.jinsphys.2019.02.008>.

440 Lacoume, S., Bressac, C., Chevrier, C., 2007. Sperm production and mating potential of males after a  
441 cold shock on pupae of the parasitoid wasp *Dinarmus basalis* (Hymenoptera: Pteromalidae). Journal  
442 of Insect Physiology, 53, 1008-1015.

443 Ma, C.S., Ma, G., Pincebourde, S., 2021. Survive a warming climate: insect responses to extreme high  
444 temperatures. Annual Review of Entomology, 66, 163-184. [https://doi.org/10.1146/annurev-ento-](https://doi.org/10.1146/annurev-ento-041520-074454)  
445 [041520-074454](https://doi.org/10.1146/annurev-ento-041520-074454).

446 Neven, L.G., 2000. Physiological responses of insects to heat. Postharvest Biology and Technology,  
447 21, 103-111.

448 Nguyen, T.M., Bressac, C., Chevrier, C., 2013. Heat stress affects male reproduction in a parasitoid  
449 wasp. Journal of Insect Physiology, 59, 248-254.

450 Polgár, L.A., Darvas, B., Völkl, W., 1995. Induction of dormancy in aphid parasitoids: implications for  
451 enhancing their field effectiveness. Agriculture, Ecosystems & Environment, 52, 19-23.  
452 [https://doi.org/10.1016/0167-8809\(94\)09003-P](https://doi.org/10.1016/0167-8809(94)09003-P).

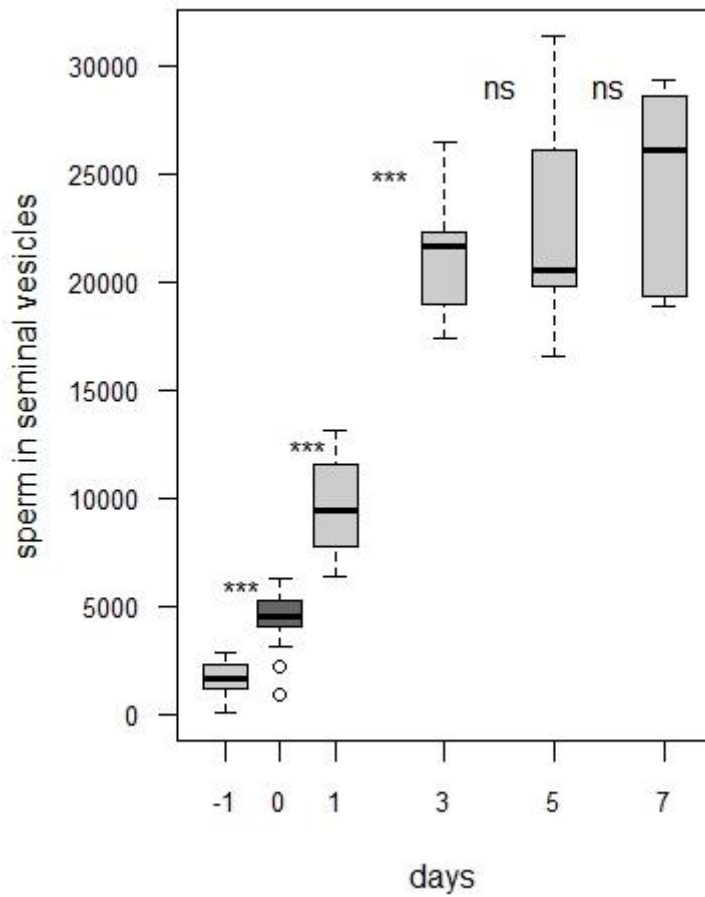
453 Quicke, D.J.L., 1997. Parasitic Wasps. Chapman & Hall, London, UK.

454 Rull, J., Lasa, R., Guillén, L., Aluja, M., 2019. The effect of winter length on duration of dormancy and  
455 survival of *Rhagoletis completa* (Diptera: Tephritidae) and associated parasitoids from northeastern  
456 Mexico. Journal of Insect Science, 19, 7. doi: 10.1093/jisesa/iez034.

457 Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A., 2019.  
458 The impact of climate change on fertility. Trends in Ecology & Evolution, 34, 249–259.  
459 <https://doi.org/10.1016/j.tree.2018.12.002>

460 Zhang, W., Zhao, F., Hoffmann, A.A., Ma, C.-S., 2013. A single hot event that does not affect survival  
461 but decreases reproduction in the diamondback moth, *Plutella xylostella*. PLoS One, 8.  
462 <https://doi.org/10.1371/journal.pone.0075923>

463

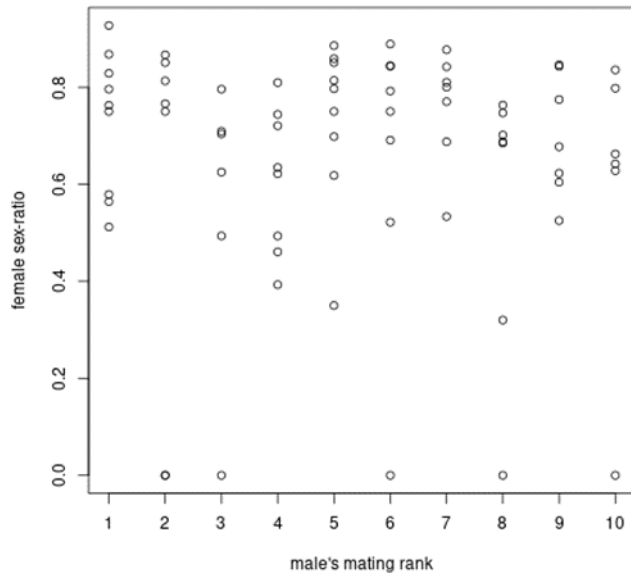


465

466 Figure 1. Sperm in seminal vesicles along days in *Cotesia typhae*. Day of emergence is 0. All males  
 467 were virgin. Tukey tests significances for consecutive ages are indicated as \*\*\* when  $P < 0.001$  and ns  
 468 when  $p > 0.05$ . Numbers are respectively  $n = 25, 30, 30, 17, 19, 6$ .

469

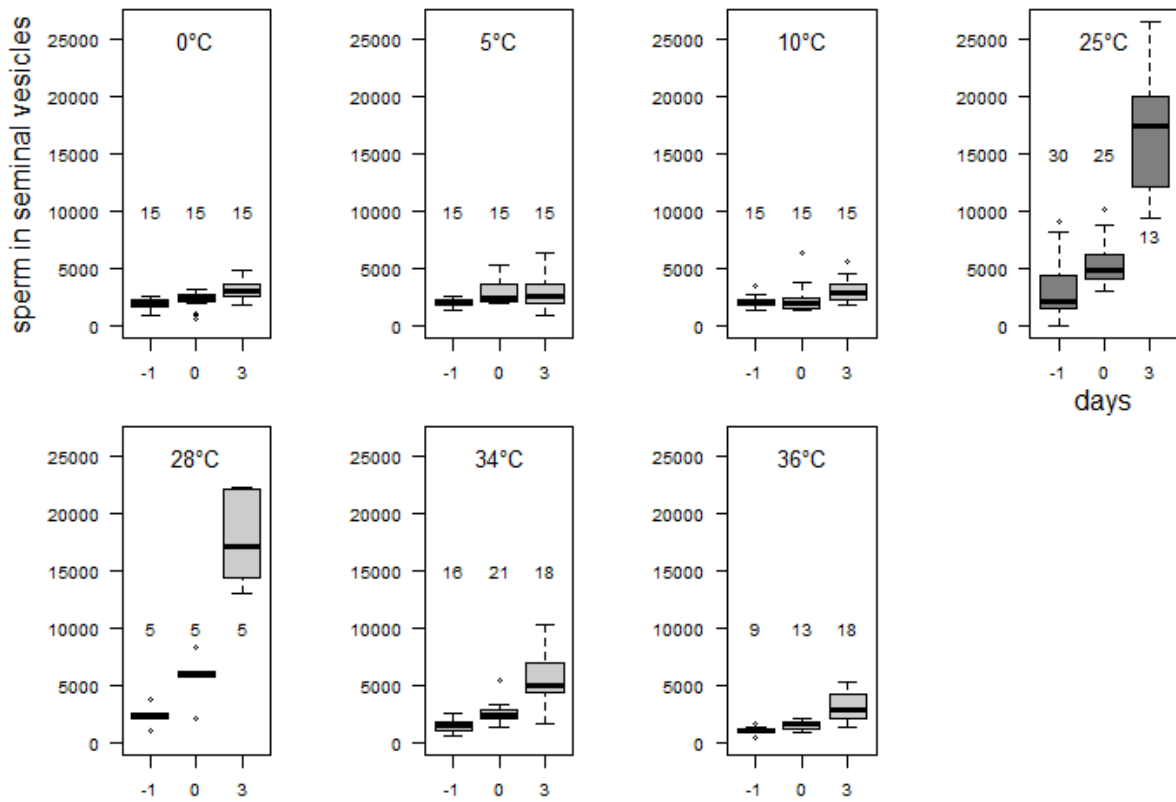




470

471 Figure 2. Female sex-ratio (proportion of females in the offspring) according to male's mating rank,  
 472 statistics are in the text.

473



475

476 Figure 3. Sperm in seminal vesicles of virgin males after temperature treatments at pupal stage.

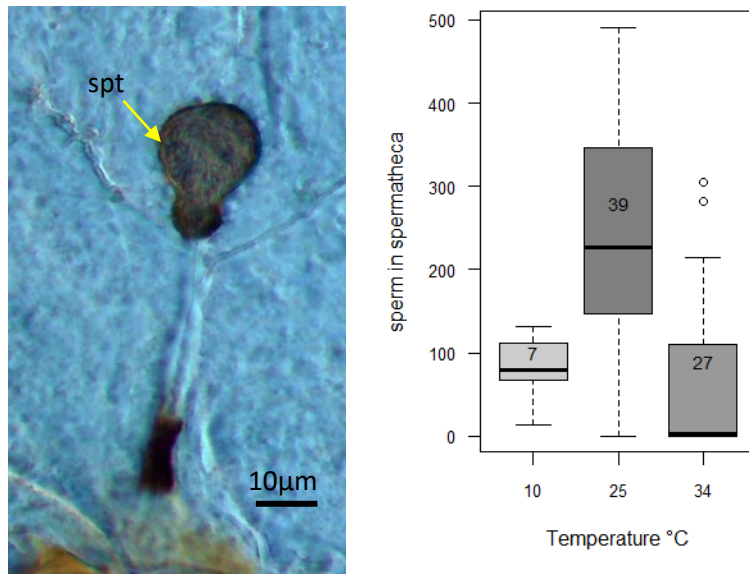
477 Pupae were exposed to 0, 5 or 10 °C for 7 days, then replaced back at 25-27°C until emergence.

478 Others were placed at 25, 28, 34 or 36 °C for 1 day and then replaced back at 25-27°C until

479 emergence. Sperm were counted one day before emergence (-1 day), the day of emergence (0 day)

480 or 3 days after emergence (3 days). Sample sizes are on graphs, statistics are in the text.

481



483

484 Figure 4. Spermatheca of *C. typhae*. 3a. spermatheca under phase contrast at a x100 objective. Spt=  
 485 sperm reservoir. 3b. Sperm counted in spermathecae of once-mated females with one male treated  
 486 at the pupal stage at either 10°C for 7 days, 25-27°C (control) or 34°C during 1 day. Numbers in boxes  
 487 indicate the number of females.

488

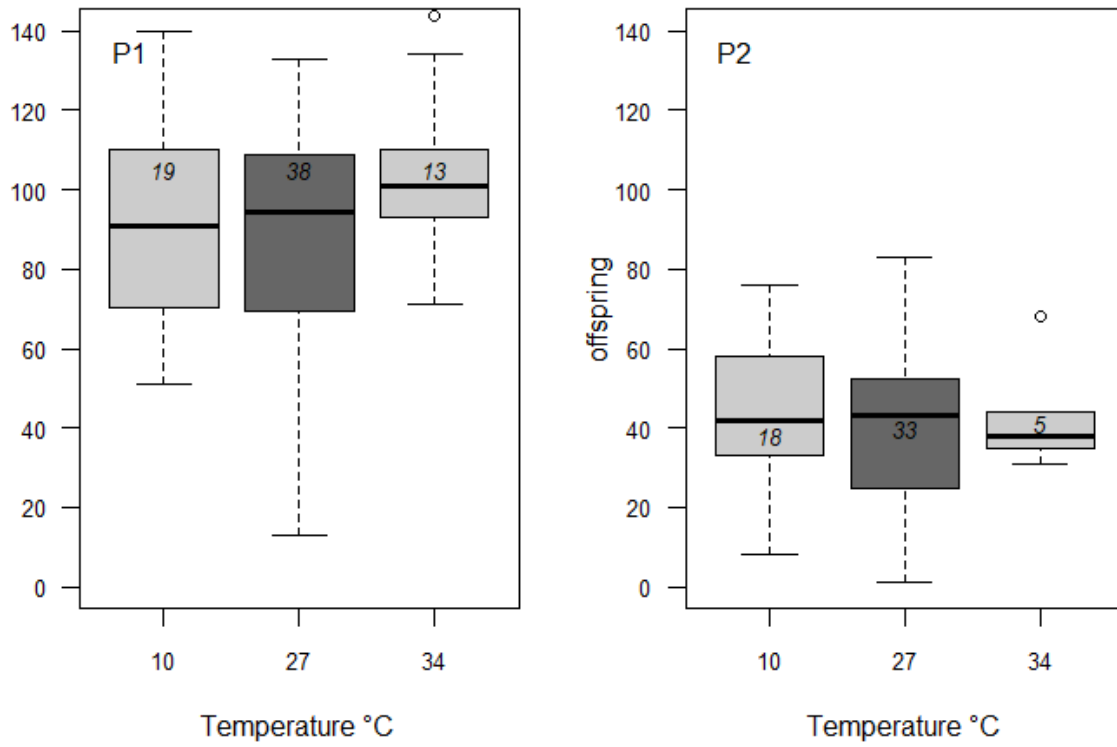
Male treatment	Host 1 P1					Host 2 P2				
	Host development	Host death	cocoons	No female N and %	total	Host development	Host death	cocoons	No female N and %	total
10 °C	3		19	10 – 53%	22	3		18	8 – 44%	21
27 °C	7	2	38	7 – 18%	47	9		33	4 – 12%	42
34 °C	3	1	13	9 – 69%	17	9	1	5	3 – 60%	15

490

491 Table 1. Fate of the stings of females of *C. typhae* mated with males that have undergone a cold or  
 492 hot period during the pupal stage. Host development means that parasitism was not successful, host  
 493 death occurred in some cases, cocoons are observed when larvae emerged from the host after a  
 494 complete development. The number and percentage of clutches containing no female offspring is  
 495 also provided as an indication of a failure in sperm transfer.

496

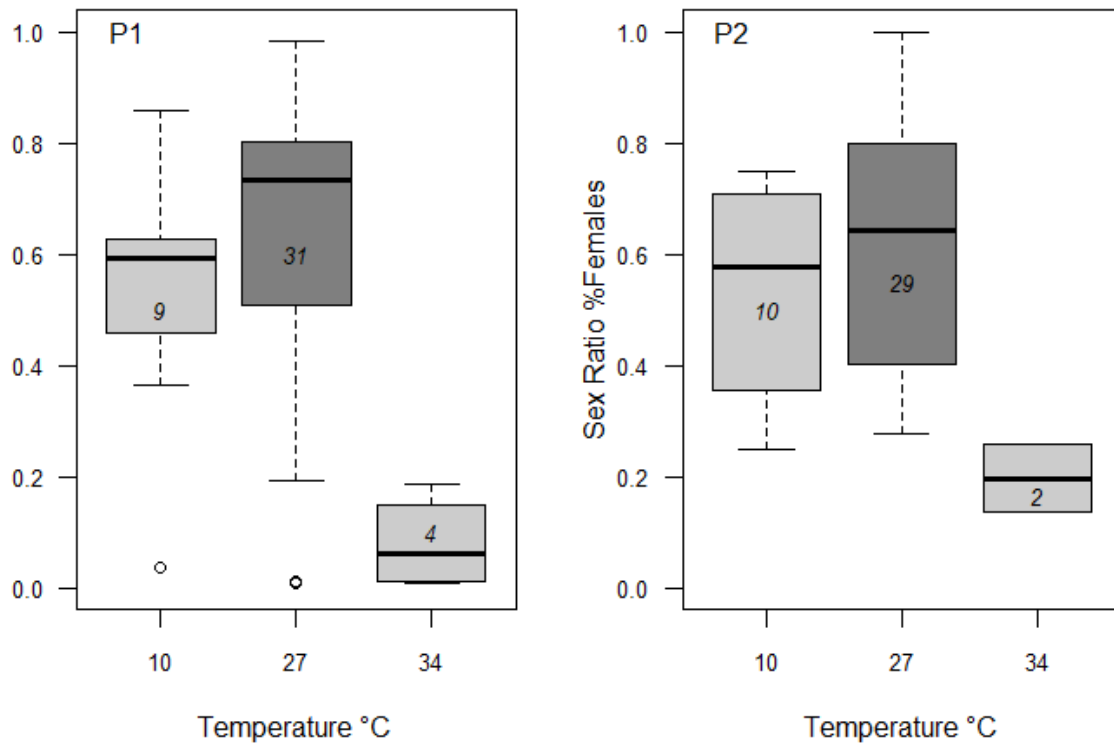
497



499

500 Figure 5. Offspring output of *C. typhae* once-mated females with one male treated at the pupal stage  
 501 at either 10°C for 7 days, 25-27°C (control) or 34°C for 1 day. P1 is the first host, P2 the second,  
 502 numbers are in bars. There is no difference according to the state of the reproductive male (P1,  
 503 ANOVA  $F_{2,49}=0.9$ ,  $p=0.4$ ; P2, ANOVA  $F_{2,49}=1.05$ ,  $p=0.36$ ).

504



505

506 Figure 6. Sex-ratio, as females / total offspring, of *C. typhae* once-mated females with one male  
 507 treated at the pupal stage at either 10°C for 7 days, 25-27°C (control) or 34°C during 1 day. P1 is the  
 508 first host, P2 the second. Numbers are in bars. Sex ratios are statistically different for P1 (Kruskal  
 509 Wallis Chisq=30.2, df=2, p<0.001) and P2 (Kruskal Wallis Chisq=19.2, df=2, p<0.001). Data do not  
 510 include clutches containing no female offspring (see table 1). Sex ratios do not differ between first  
 511 (P1) and second (P2) egg-laying, for 10°C, Mann-Whitney, U=0.75, df=18, p=0.23; for 25-27°C, t test,  
 512 t=0.51, df=60, p=0.30; for 34°C, Mann-Whitney, U=0.64, df=5, p=0.27.

513