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Influence of temperature on patch residence time in parasitoids: physiological and behavioural mechanisms

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1 **Influence of temperature on patch residence time in parasitoids:**
2 **Physiological and behavioral mechanisms.**

3 **Running title:** Influence of temperature on patch residence time

4

5 **Joffrey Moiroux ^{a,b}, Philippe Louâpre ^c, Paul K. Abram ^a, Maryse Barrette ^b, Jacques**
6 **Brodeur ^a and Guy Boivin ^b**

7

8 ^a Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université
9 de Montréal, 4101 rue Sherbrooke Est, Montréal, Québec, Canada, H1X 2B2.

10 ^b Centre de Recherche et de Développement en Horticulture, Agriculture et Agroalimentaire
11 Canada, 430 boul. Gouin, Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6.

12 ^c Biodiversity Research Centre, Earth and Life Institute, Université Catholique de Louvain,
13 Place de l'Université 1, 1348 Louvain-La-Neuve, Belgium.

14

15 Contact : joffrey.moiroux@gmail.com. Tel : +1 514 343 6111 ext. 82121

16

17 **ABSTRACT.**

18 1. Patch time allocation has received much attention in the context of optimal foraging
19 theory, including the effect of environmental variables. We investigated the direct role
20 of temperature on patch time allocation by parasitoids through physiological and
21 behavioral mechanisms and its indirect role via behavioral defences of the hosts.

22 2. We compared the influence of foraging temperature on patch residence time between
23 an egg parasitoid, *Trichogramma euproctidis*, and an aphid parasitoid, *Aphidius ervi*
24 that attacks hosts able to actively defend themselves.

25 3. Patch residence time decreased with an increase in temperature in both species. The
26 increased activity levels with warming, as evidenced by the increase in walking speed,
27 partly explained these variations but other mechanisms were involved. In *T.*
28 *euproctidis*, the ability to externally discriminate parasitized hosts decreased at low
29 temperature, resulting in a longer patch residence time. For *A. ervi*, we observed that
30 aphids frequently escaped at intermediate temperature and defended themselves
31 aggressively at high temperature but displayed few defence mechanisms at low
32 temperature. These defensive behaviors resulted in a decreased patch residence time
33 for the parasitoid, and partly explained the fact that *A. ervi* remained for a shorter time
34 at the intermediate and high temperatures than at the lowest temperature.

35

36 **Keywords:** patch time allocation, host-parasitoid interaction, climate, discrimination ability,
37 sex allocation, anti-predator behaviors.

38

39 INTRODUCTION

40 Resources such as food, mate or hosts are usually heterogeneously distributed in the
41 environment and their exploitation requires time. One of the main decisions an organism faces
42 when foraging is how to manage residence time between visited patches in order to maximize
43 fitness yield (Wajnberg, 2006). Patch time optimization received much attention in the
44 context of optimal foraging, and was first addressed by Charnov (1976) in his Marginal Value
45 Theorem (MVT). The main assumption of the MVT is that a forager should leave a depleted
46 patch when the current rate of gain falls below the average rate it would come across in the
47 environment, thus maximizing the long term rate of fitness gain. This model is one of the
48 most influential in behavioral ecology but it has been subjected to criticism as it assumes that
49 foragers are omniscient, and does not take into account proximate behavioral mechanisms
50 involved in patch time allocation (van Alphen & Bernstein, 2008).

51 Insect parasitoids have been widely used as model organisms to study patch time
52 allocation as their hosts are often patchily distributed and their foraging behavior directly
53 influences progeny production. Over the years, many authors investigated behavioral
54 mechanisms involved in patch time allocation (e.g., Waage, 1979; Driessen *et al.*, 1995) and
55 have shown that parasitoids can adapt patch residence time to a wide variety of environmental
56 variables (for a review, see Wajnberg, 2006), among them biotic factors such as presence of
57 competitors (Wajnberg *et al.* 2004; Goubault *et al.*, 2005; Le Lann *et al.*, 2010) and host
58 kairomones (Li *et al.*, 1997; Louâpre & Pierre, 2012) or abiotic factors such as photoperiod
59 during development (Roitberg *et al.*, 1992) and barometric pressure (Roitberg *et al.*, 1993).

60 Despite the considerable influence of temperature on life history, physiological and
61 behavioral traits of insects (Huey & Kingsolver, 1989; Nylin & Gotthard, 1998; Angilletta *et*
62 *al.*, 2004), its influence on patch time allocation has rarely been addressed. Temperature may

63 directly affect patch residence time through physiological or behavioral mechanisms. Firstly,
64 walking speed, search rate, handling time and related behaviors are known to change with
65 temperature in ectotherms because of its influence on metabolic rate (see the Metabolic
66 Theory of Ecology, Brown *et al.*, 2004; Moiroux *et al.*, 2012). Usually, walking speed and
67 search rate follow a hump-shaped relationship with temperature in insects (Sentis *et al.*,
68 2012). It is thus likely that patch residence time would vary with temperature as a direct
69 consequence of its influence on the speed of movements and behaviors. Secondly, behavioral
70 mechanisms may have been selected in response to temperature. To our knowledge, only
71 Amat *et al.* (2006) have convincingly addressed this issue in a parasitoid. They observed that
72 a drop in temperature before foraging increased patch residence time and that this
73 environmental cue was more important than foraging temperature itself. The authors proposed
74 that thermal variations may be perceived as a signal of unfavourable weather conditions and
75 that searching for a new patch would increase mortality risk or physiological costs. Finally,
76 multi-objective behavioral mechanisms related to patch time allocation may be adopted by
77 animals in response to temperature. Wajnberg (2012) clearly showed that sex allocation
78 influenced patch time allocation in the egg parasitoid *Trichogramma chilonis* as laying a son
79 did not change its patch residence time while laying a daughter decreased its patch time
80 allocation. Considering that haplodiploid parasitoids can adapt sex allocation to their foraging
81 temperature (Moiroux *et al.*, 2014), we may thus expect that females would also change their
82 patch time allocation according to sex allocation.

83 Temperature may also indirectly influence patch time allocation via its effect on the nature
84 and frequency of host defences (e.g., Miyatake *et al.*, 2008; Ma & Ma, 2012). For example,
85 when exposed to alarm pheromone, pea aphids *Acyrtosiphon pisum* are less likely to drop
86 from the feeding site to the ground when the environment is hot and dry than when it is more
87 benign (Dill *et al.*, 1990). Temperature may thus affect patch time allocation through its

88 influence on host defences since parasitoids can change patch time allocation as a response to
89 host defence behaviors. This was for example observed for the ichneumonid *Diadegma*
90 *semiclausum*, which remains longer on a patch when attacked hosts temporarily leave (Wang
91 & Keller, 2003).

92 The aim of this study was to investigate both the direct and indirect effects of temperature on
93 patch time allocation in parasitoids. We compared the influence of temperature on patch
94 residence time of two different parasitoid species exploiting hosts that exhibit or not defensive
95 behaviors: (i) the parasitoid *Trichogramma euproctidis* Girault (Hymenoptera:
96 Trichogrammatidae), which attacks eggs of the lepidopteran species *Ephestia kuehniella*
97 Zeller (Lepidoptera: Pyralidae) and for which we were able to distinguish male egg deposition
98 from female egg deposition by observation (Moiroux *et al.* 2014) to study influence of sex
99 allocation, and (ii) the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae) which
100 attacks potato aphids, *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae), that are
101 able to actively defend themselves. For this parasitoid species, we were unable to measure sex
102 allocation. Both parasitoid species were maintained at their optimal temperature and placed at
103 low and high temperatures close to their thermal limits before foraging. Their patch residence
104 time and foraging behaviors were then recorded. We also measured thermal variations in
105 walking speed for *T. euproctidis* as a proxy of physiological changes, while data were already
106 available from Gilchrist (1996) for *A. ervi*. The nature and frequency of defensive behaviors
107 displayed by aphids attacked by *A. ervi* was also measured. We expected that patch time
108 allocation should be directly influenced by temperature for both species as a consequence of
109 changes in walking speed, and indirectly via its effect on sex allocation in *T. euproctidis*
110 (Moiroux *et al.* 2014) and via its effects on behavioral defences of hosts in *A. ervi*.

111

112 **MATERIALS & METHODS**

113

114 *Trichogramma euproctidis*

115 Trichogrammatidae wasps are small endoparasitoids of eggs of several lepidopteran species.
116 The *T. euproctidis* strain used in this study originates from individuals sampled in Egypt and
117 was maintained at 24 ±1°C, 50% RH, and LD 16:8 h on cold-killed eggs of *Ephestia*
118 *kuehniella*.

119

120 **Patch residence time.** Parasitized eggs were taken from the *T. euproctidis* colony and
121 isolated in 300µL Beem[®] polyethylene capsules with a drop of honey. The next morning,
122 newly emerged females were mated once with a virgin male, stored at 24°C, and transferred to
123 a climate room at either 14, 24 or 34°C one hour before observation. The first and last
124 temperatures are close to the species thermal limits but do not cause any deleterious effects on
125 walking activity (see results on walking activity). After this acclimatization period, females
126 were offered, at the same temperature, 20 cold-killed *E. kuehniella* eggs, placed side by side
127 in 4 lines of 5 eggs on a filter paper disc. The experimental arena was delimited by the plastic
128 end piece of a camera (Ø20 mm) covered with fluon to prevent females from climbing up the
129 side. The number and duration of the following behaviors were recorded for 30 females per
130 temperature with a camera (Dino-lite Pro AM413ZT, x 60) and analyzed using The Observer
131 XT software (Noldus, Wageningen, The Netherlands): entering the patch, leaving the patch,
132 walking, host feeding, drumming the host with antennae, drilling, egg male deposition, egg
133 female deposition, host rejection, and resting. Egg deposition was distinguished from drilling
134 by positioning of the abdomen, which was perpendicular to the host when drilling and parallel
135 during egg deposition. Egg female deposition was distinguished from egg male deposition by

136 the presence of a pause during oviposition sequence (Suzuki *et al.* 1984). Host rejection
137 consisted of drilling not followed by egg deposition. Walking was recorded when a female
138 was moving and did not mark a pause while drumming hosts with antennae was noted when a
139 female was on an egg and touched it repeatedly with its antennae. We did not consider host
140 rejection with antennae, as it was sometimes very fast, especially at high temperature, and
141 difficult to distinguish from walking on eggs. Patch residence time was measured from the
142 time the female entered the patch until she remained inactive for more than 30 minutes or
143 walked off the patch for more than 3 minutes at 34°C, 4min45s at 24°C and 7min30s at 14°C.
144 These durations are proportional to speed variation due to temperature measured in the
145 experiment on walking speed described below. Additional analyses were performed
146 considering a similar threshold, i.e. females walked off the patch for more than 3 minutes for
147 the three temperatures, and the results led to the same conclusion in both cases.

148

149 **Walking speed.** Newly emerged (<24 h), unfed females were placed individually in an empty
150 Petri dish (Ø92 mm) on a light table at 10, 15, 20, 25, 30 or 35±1°C. The movement of 15
151 females per temperature was video-recorded (Camera Panasonic WV-BD400) for 300
152 seconds and then analyzed with a program developed by Vigneault *et al.* (1997). The contrast
153 between individuals and the light table was used to track females and the program calculated
154 the mean walking speed (mm/sec), deduced from the distance traveled in 300 seconds minus
155 duration of stops.

156

157

158 *Aphidius ervi*

159 **Insect colonies.** *Aphidius ervi* is a solitary koinobiont parasitoid of several aphid species. A
160 few weeks prior to the tests, an *A. ervi* colony was established with individuals bought from
161 BioBest Canada (Leamington, Canada). Parasitoids were reared in cages on the potato aphid
162 *M. euphorbiae* at $20\pm 1^{\circ}\text{C}$, $60\pm 10\%$ RH, 16L: 8D photoperiod. The aphid colony was initiated
163 with individuals collected from potato fields in the vicinity of Québec City, Canada. This
164 colony was maintained on potato plants, *Solanum tuberosum* Linnaeus (Solanales: Solanaceae),
165 cultivar “Norland” under the same conditions.

166

167 **Patch residence time.**

168 Parasitized aphids were reared at $20\pm 1^{\circ}\text{C}$ on potato plants and isolated in gelatine capsules
169 once they mummified. After emergence, each female parasitoid was kept with a male at
170 $20\pm 1^{\circ}\text{C}$ for 24 hours in a Petri dish containing moistened cotton and honey. This period
171 allowed females to mature eggs (Le Lann *et al.*, 2010). The next day, their foraging behavior
172 was observed in climate chambers at 12, 20 or 28°C . The first and last temperatures are close
173 to *A. ervi* thermal extremes but do not induce any lethal effects or deleterious effects on
174 walking activity (according to Gilchrist, 1996). One hour before the observation, a potato leaf
175 (\varnothing 4cm approximately) was taped on a piece of paper and 60 apterous aphids were placed on
176 it, 15 from each larval instar (L1 to L4). A range of different instars were exposed to *A. ervi*
177 females because their defensive behaviors change with age (Barrette *et al.*, 2010). Prior to the
178 test, parasitoid females were acclimatized to the experimental temperature for one hour.
179 Females were then introduced in the experimental arena (\varnothing 26cm, h 8.5 cm) and the following
180 behaviors were recorded with a camera (Panasonic WV-BD400) on 25 females per
181 temperature and analyzed with The Observer XT software (Noldus, Wageningen, The
182 Netherlands): entering the patch, leaving the patch, walking, successful oviposition, failed

183 oviposition, host rejection and resting. A successful oviposition was recorded when the
184 parasitoid abdomen came into contact with the aphid while a failed oviposition was recorded
185 when a parasitoid initiated an attack but failed to touch the aphid with its abdomen because of
186 aphid behavioral defences. Host rejection was recorded when a female parasitoid came into
187 contact with a motionless aphid and did not attack it. Both the number and duration of these
188 behaviors were recorded, taking into account host instar which was evaluated visually by
189 aphid body size. Patch residence time was measured from the time the female entered the
190 patch until she left it for more than one minute at 28°C, 2 minutes at 20°C and 6 minutes at
191 12°C or stopped any activity for more than 15 minutes. These durations are proportional to
192 speed variations with temperature measured by Gilchrist (1996). Additional analyses were
193 performed considering a similar threshold, i.e. females left the patch for more than 1 minute
194 for the three temperatures, and the results led to the same conclusion in both cases.

195 During this test, we also recorded aphid anti-predator behaviors, i.e. kicking, body jerking, or
196 escaping from the patch. Kicking was recorded when an aphid extended one of its leg, usually
197 a hind leg, and shook it up and down. Body jerking consisted of spasmodic horizontal
198 movements of the abdomen. Escaping the patch was recorded when an aphid walked away
199 after an attack and left the potato leaf. These behaviors were only recorded when aphids were
200 attacked by the parasitoid.

201 For this parasitoid species, we were not able to study the influence of sex allocation on patch
202 residence time. In *Aphidius* parasitoids, sex allocation can not be determined by observation.
203 In his paper on multi-objective behavioural mechanisms, Wajnberg (2012) observed the sex
204 of emerging parasitoids to assess sex allocation during oviposition. However the temperature
205 is known to affect sex ratio via physiological constraints on egg fertilization in haplodiploid
206 parasitoids (Moiroux *et al.* 2014), resulting in differences between sex allocation and realized

207 sex ratio. Observation of the sex of emerging parasitoids was thus not reliable to evaluate sex
208 allocation in our experiment.

209

210 **Statistical analyses.**

211 Patch residence time was analyzed using a Cox proportional hazard model (Cox, 1972;
212 Tenhumberg *et al.*, 2001). Such model is classically used to analyse censored data such as
213 patch duration (Wajnberg *et al.*, 1999). It determines a patch-leaving tendency for a foraging
214 parasitoid, *i.e.* the probability per unit of time that a female leaves the patch, providing that
215 she is still on it. It is formulated in terms of hazard ratio; a hazard ratio above one indicates an
216 increase in the female's patch-leaving tendency and a hazard ratio below one indicates a
217 decrease in the patch-leaving tendency. For *T. euproctidis*, temperature was considered as
218 fixed factor, while the number of male ovipositions, number of female ovipositions, number
219 of host rejections, number of host feeding, resting periods and temporary exit from the patch
220 were included as time-dependant covariates. The interaction between temperature and the
221 number of male and female ovipositions or with host rejections were also considered. For *A.*
222 *ervi*, temperature, considered as a fixed factor, total number of ovipositions, number of
223 ovipositions per host larval instar, number of failed ovipositions, number of host rejections,
224 number of aggressive behaviors (*i.e.*, kicking + jerking) and of escaping aphids, number of
225 temporary exits from the patch were included in the model, as were interactions between
226 temperature and the number of successful or failed ovipositions. In both Cox models, the
227 adequacy of the model was assessed by plotting deviance residuals (Therneau *et al.*, 1990).
228 The assumption that hazard rates were proportional for different values of covariates was also
229 checked following Collett (1994) and Wajnberg *et al.* (1999).

230 In addition, the influence of temperature on the total number of ovipositions, host rejections,
231 host feeding, resting and grooming periods for *T. euproctidis* were analysed using
232 Generalized Linear Models (GLM) with a Poisson error distribution. Data on sex allocation
233 have been previously published in Moiroux *et al.* (2014). The influence of temperature on the
234 proportion of time devoted to each of these behaviors and time spent walking and drumming
235 hosts with antennae was analyzed with a GLM with a Binomial error distribution. For *A. ervi*,
236 the influence of temperature on the number and duration of these behaviors is described in
237 Moiroux *et al.* (2015). The number of kicking, jerking and escaping aphids was compared
238 using a GLM with a Poisson error distribution.

239 Finally, we fitted an exponential curve to the data to investigate the effect of temperature on
240 *T. euproctidis* walking speed.

241

242 All statistical analyses were carried out using R software version 2.14.1 (R Development Core
243 Team, 2011).

244

245 RESULTS

246 *Trichogramma euproctidis*

247 **Patch residence time.** Temperature, the number of male and female ovipositions, and the
248 interaction between temperature and the number of hosts rejection significantly influenced the
249 patch residence time of *T. euproctidis* (Table 1) while host rejection, host feeding, the number
250 of temporary exits and resting periods did not. The tendency to leave the patch increased with
251 temperature (Figure 1) and following a male and female oviposition (i.e. hazard ratio > 1,
252 Table 1). It should be noted that laying a son or of daughter increased patch leaving tendency
253 by very close factors (respectively 0.558 ± 0.166 vs 0.576 ± 0.077). Host rejection increased
254 the tendency to leave the patch at intermediate and high temperature but it decreased this
255 tendency at low temperature (Figure 2). We did not observe any significant influence of the
256 interaction between temperature and the number of male (Temperature(24): $\beta = -0.243 \pm$
257 0.263 , $p = 0.666$; Temperature(34): $\beta = -0.864 \pm 0.473$, $p = 0.167$) or female ovipositions
258 (Temperature(24): $\beta = -0.047 \pm 0.217$, $p = 0.827$; Temperature(34): $\beta = -0.058 \pm 0.220$, $p =$
259 0.791) on patch leaving tendency.

260 Temperature did not influence the total number of ovipositions while the number of host
261 rejections was significantly higher at 14°C than at 24 or 34°C, and females host-fed more at
262 34°C (Table 2). Parasitoid females laid more sons at high temperature than at other
263 temperatures while no difference in sex allocation was observed between low and
264 intermediate temperatures (Moiroux *et al.*, 2014). Females foraging at 14°C spent
265 proportionally less time laying eggs ($df = , p < 0.001$), walking ($df = , p = 0.021$) and feeding
266 on hosts ($df = , p = 0.014$) than females tested at 24 and 34°C, but spent significantly more
267 time drumming host eggs with their antennae ($df = , p = 0.003$) and rejecting hosts ($df = , p <$
268 0.001) (Figure 3).

269

270 **Walking speed.** Walking speed of *T. euproctidis* increased significantly with increasing
271 temperature ($F = 118.7$, $df = 1, 87$, $p < 0.001$; Figure 4).

272

273 *Aphidius ervi*

274 **Patch residence time.** Patch residence time was significantly influenced by temperature
275 (Figure 1B), the number of total successful ovipositions, the number of escaping aphids and
276 the number of aggressive aphids, but not by other time-dependent covariates or the
277 interactions between temperature and any of the time-dependent covariates (Table 3). Females
278 remained longer in the patch after an oviposition and left earlier with increasing temperature
279 and the number of aggressive or escaping aphids.

280

281 **Aphids.** The number of kicks performed by *M. euphorbiae* ($t = 7.718$, $df = 73$, $p < 0.001$) and
282 the number of aphids jerking ($t = 3.146$, $p = 0.002$) were significantly influenced by the
283 foraging temperature. Aggressive defensive behaviors were more frequent at 28°C and less
284 frequent at 12°C (Figure 5). Foraging temperature significantly influenced the number of
285 aphids escaping from the patch when attacked ($t = 3.914$, $p < 0.001$). Escape behavior was the
286 most frequent in aphids attacked at 20°C (Figure 5).

287

288 **DISCUSSION**

289 We investigated the influence of temperature on patch time allocation in two parasitoid
290 species, considering its direct effects through physiological or behavioral mechanisms and its
291 indirect effects via behavioral defences of hosts. Our main results are that: (1) patch residence
292 time decreased as temperature increased, together with an increase in walking speed for both
293 *T. euproctidis* and *A. ervi*, (2) patch time allocation was influenced by an interaction between
294 temperature and the number of host rejections in *T. euproctidis*, and (3) the nature and
295 number of aphid behavioral defences changed with temperature and influenced patch
296 residence time in *A. ervi*.

297

298 **Patch time allocation.** *Trichogramma euproctidis* females used a decremental mechanism
299 when exploiting host patches as each oviposition increased the patch leaving tendency. This
300 decremental mechanism is consistent with the results of a comparative analysis conducted on
301 17 species of *Trichogramma* by Wajnberg *et al.* (2003). An increased tendency to leave a
302 patch after oviposition is expected when resources are uniformly distributed over patches
303 (Iwasa *et al.*, 1981). However, this hypothesis cannot be confirmed as no reliable distribution
304 data are available for hosts of *Trichogramma* parasitoids (Wajnberg *et al.*, 2003).

305 On the contrary, patch time allocation increased due to an oviposition in *Aphidius ervi*. This
306 incremental mechanism (Waage, 1979) has been observed in many parasitoid species (for a
307 review, see Wajnberg 2006) including a close and sympatric species, *Aphidius rhopalosiphii*
308 (Outreman *et al.*, 2005). Such behavior is expected when organisms face a large variance in
309 patch quality (Iwasa *et al.*, 1981), as observed for aphid colonies in the field (e.g., Ward *et al.*,
310 1986).

311

312 **Patch time allocation and temperature.** In both parasitoid species, patch residence time
313 decreased sharply when temperature increased. This result contrasts with a study by van
314 Roermund *et al.* (1994) who did not observe any influence of temperature on patch time
315 allocation in the whitefly parasitoid *Encarsia formosa*. It should be noted that in this last
316 paper, parasitic wasps were tested on patches without hosts. These unnatural conditions likely
317 explain the absence of parasitoid response to temperature.

318 An increase in female parasitoid activity with warming, as evidenced by the increase in
319 walking speed in *T. euproctidis* (this study) and *A. ervi* (Gilchrist, 1996), would likely
320 modulate patch residence time. The higher the temperature, the faster females perform the
321 component behaviors that make up foraging up to an optimum, as predicted in the context of
322 the Metabolic Theory of Ecology (Brown *et al.*, 2004). In *T. euproctidis* and *A. ervi*, walking
323 speed increased on average 1.7 times between 24 and 34°C and 20 and 28°C, respectively,
324 while patch residence time and duration of the different behaviors recorded (antennation,
325 drilling, egg deposition) decreased 1.7 times. This result suggests a simple physiological
326 change associated with temperature. However, while the walking speed increased 1.6 times
327 from 14 °C to 24 °C and 2.5 times from 14 °C to 34 °C in *T. euproctidis*, patch residence time
328 decreased 2.6 and 8.7 times, respectively. In *A. ervi*, walking speed increased 4.1 times from
329 12 to 20°C and 6.9 times from 14 to 34°C while patch residence time decreased 5 and 8.6
330 times, respectively. These differences suggest that in both species walking speed alone does
331 not explain the increase in patch time allocation at the lowest temperature. From our results,
332 different mechanisms may be involved for each species: a decrease in external discrimination
333 ability in *T. euproctidis* and variations in nature and frequency of host defensive behaviors in
334 *A. ervi*. These mechanisms were observed in parasitoids and hosts taken from the lab but field
335 populations living under changing environment may respond differently than these strains.

336 At the lowest temperature, the number of host rejections (i.e. a drilling not followed by
337 egg deposition) by *T. euproctidis* females was higher than at other temperatures. Parasitized
338 eggs were thus mainly rejected after perforation of the chorion by the ovipositor but females
339 were unable to reject them while drumming with their antennae, unlike at 24 or 34°C. These
340 results indicate that a low temperature during oviposition affected the external discrimination
341 ability of *T. euproctidis* females, which probably contributed to the increased patch residence
342 time recorded at 14°C. Overall, the same number of eggs was parasitized by females across
343 tested temperatures, but it took more time to exploit the patch at low temperature. Several
344 non-exclusive hypotheses can explain the decrease in external discrimination ability. First, the
345 amount or composition of the marking pheromone produced by a female and left on the host
346 after oviposition (Roitberg & Mangel, 1988) may be changed at low temperature, as observed
347 for the marking pheromone produced by beewolf males, *Philanthus triangulum*, to mark their
348 territories (Roeser-Mueller *et al.*, 2010). Secondly, the receptivity of antennae to chemical
349 signals or their integration in the olfactory lobe may also be affected, as suggested for
350 European corn borer males, *Ostrinia nubilalis*, exposed to female sex pheromone at different
351 temperatures (Hilker & McNeil, 2008). An effect of low temperature on the nervous system,
352 especially on learning ability, was also proposed by van Baaren *et al.* (2005) to account for
353 the absence of external discrimination ability in cold-stored females of the mymarid *Anaphes*
354 *victus*.

355 This negative effect of low temperature on external discrimination ability probably explains
356 that we observed a significant influence of the interaction between temperature and host
357 rejection on patch residence time in *T. euproctidis*. At intermediate and high temperature,
358 *Trichogramma* females left earlier the patch after host rejection. This decremental mechanism
359 is assumed to be adaptive and has been commonly reported in parasitoids (Wajnberg, 2006)
360 including species from the *Trichogramma* genus (Wajnberg *et al.*, 2003). At low temperature,

361 we observed an incremental mechanism associated with host rejection. This behavioral
362 mechanism has only been reported in one parasitoid species (Nelson & Roitberg, 1995) and is
363 probably the consequence of deleterious effect of temperature on sensory structures in our
364 experiment. *A. ervi* did not suffer as much as *T. euproctidis* from low temperature. It is likely
365 that interspecific variations or local adaptations of reproductive behaviors at low temperature
366 exist since oviposition does not seem to be negatively affected by temperatures close to 0°C
367 in some parasitoids (Meiners *et al.*, 2006). Size differences between the species might for
368 example explain these differences. Due to their small size and thermoregulatory constrains,
369 egg parasitoids might be physiologically more affected by changes in temperature than the
370 larger aphid parasitoids.

371 We hypothesised that temperature may influence patch time allocation via its effect on sex
372 allocation (Wajnberg, 2012) since in *T. euproctidis*, Moiroux *et al.* (2014) observed that the
373 number of sons laid by a female was greater at high temperature than at low and intermediate
374 temperatures. Laying a son or of daughter increased patch leaving tendency by very close
375 factors. This result contrasts with the paper of Wajnberg (2012) who observed in *T. chilonis*
376 that laying a son did not influence patch residence time while it decreased with oviposition of
377 a daughter. Our result, as well as the absence of interaction between male or female egg
378 deposition and temperature, imply that differences in patch residence time with temperature
379 could not be explained by differences in sex allocation.

380

381 In *A. ervi*, the nature and frequency of defensive behaviors displayed by aphids
382 changed with temperature. They frequently defended themselves by kicking and jerking at the
383 highest temperature while they tended to escape at the intermediate temperature. This
384 observation is consistent with results on pea aphids, *Acyrtosiphon pisum*, which are less
385 likely to drop from their feeding site when the environment is hot and dry than when it is

386 more benign (Dill *et al.*, 1990). Aphids may be less prone to leave a leaf when temperature is
387 high as they need more resources to compensate for the increased metabolic rate (Brown *et*
388 *al.*, 2004) and should actively defend against parasitoids instead of escaping. Aphid
389 aggressive behaviors resulted in an increase in patch leaving tendency for the parasitoid *A.*
390 *ervi*. This effect may explain why the patch residence time of aphid parasitoids was shorter at
391 28°C than expected from the change in walking speed when compared to the lowest
392 temperature, at which aphids rarely defended. Since aggressive host behavioral defences
393 increases injury risk for parasitoids, it is likely that the optimal behavior would be to search
394 for another patch with less aggressive hosts. To our knowledge, this is the first experimental
395 evidence that such aggressive behavioral defences influence patch time allocation in insect
396 natural enemies. We also observed that patch residence time decreased with the number of
397 aphids leaving the patch when exposed to a parasitoid. This last result contrasts with
398 observation on the ichneumonid parasitoid *Diadegma semiclausum* that remained longer on a
399 patch when hosts temporally left after attack (Wang & Keller, 2003), waiting for the
400 discovered host to climb back to the leaf (Wang & Keller, 2002). *Aphidius ervi* instead left the
401 patch earlier, probably because females perceived a decrease in patch quality (e.g., Boivin *et*
402 *al.*, 2004; Outreman *et al.*, 2001). This result may explain why *A. ervi* remained shorter than
403 expected at the intermediate temperature compared to the lowest temperature, since aphids
404 escaped more frequently at intermediate temperature.

405

406 **Conclusion.** Patch time allocation is influenced by several environmental variables, including
407 temperature. We present evidence that foraging temperatures directly influences patch time
408 allocation in parasitoids, mainly through physiological mechanisms, and indirectly through
409 changes in the nature and frequency of host behavioral defences. In the next decades,
410 temperature variance and the frequency of extreme climatic events are expected to increase

411 (IPCC 2007). From our results, we could predict that some species will be more affected than
412 others, as a change in temperature modified the influence of an host rejection on patch leaving
413 tendency in *T. euproctidis* but not in *A. ervi*. In parasitoid species attacking hosts that actively
414 defend themselves, the influence of the predicted climate change on patch time allocation
415 might be more closely related to the induced changes in host behavioral defences than the
416 direct effect of temperature on the parasitoid itself. This result suggests that both parasitoids
417 and hosts should be considered when investigating the role of climate on patch residence
418 time.

419

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425

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613

Table 1. Estimated regression coefficients (β) and their standard errors, and hazard ratios ($\exp(\beta)$) obtained for covariates of a Cox proportional hazard model relating patch residence time and behaviors observed in *T. euproctidis*.

	$\beta \pm \text{s.e.}$	$\exp(\beta)$	z	p-value
Temperature(24)	2.189 \pm 0.765	8.933	2.862	0.004
Temperature(34)	2.654 \pm 0.949	14.208	2.797	0.005
Male oviposition	0.558 \pm 0.166	1.747	3.496	< 0.001
Female oviposition	0.576 \pm 0.077	1.779	7.407	< 0.001
Host rejection	-0.059 \pm 0.035	0.942	-1.686	0.091
Temperature(24) : rejection ^a	0.136 \pm 0.062	1.146	2.201	0.027
Temperature(34) : rejection ^a	0.754 \pm 0.184	2.126	4.088	< .001

^a Interaction

Table 2. Total number of ovipositions, host rejections, host feeding and resting periods measured on *T. euproctidis* females allowed to parasitize 20 cold-killed *E. kuehniella* eggs at three temperatures. Host rejection refers to a drilling not followed by egg deposition. Mean \pm SE.

Temperature ($^{\circ}$ C)	Oviposition	Host rejection	Host feeding	Resting period
14	18.60 \pm 0.23	15.27 \pm 1.02	1.62 \pm 0.25	0.80 \pm 0.19
24	18.63 \pm 0.33	3.45 \pm 0.14	1.83 \pm 0.23	0.40 \pm 0.15
34	18.57 \pm 0.33	2.17 \pm 0.4	2.77 \pm 0.50	0.53 \pm 0.19
t value (df = 89)	-0.095	-10.660	2.966	-1.255
p-value	0.924	<0.001	0.003	0.213

616 **Table 3.** Estimated regression coefficients (β) and their standard errors, and hazard ratios ($\exp(\beta)$) for
 617 significant covariates of a Cox proportional hazard model relating patch residence time, temperature and
 behaviors of *A. ervi* females allowed to attack four stages of *M. euphorbiae*.

	$\beta \pm \text{s.e.}$	$\exp(\beta)$	z	p-value
Temperature(20)	1,817 \pm 0.528	6,153	2,823	0.024
Temperature(28)	2,344 \pm 0.712	10,425	3,046	0.009
Oviposition	-0,348 \pm 0.104	0,706	-2,370	0.035
Aggressive aphids	0,333 \pm 0.092	1,395	2,972	0.016
Escaping aphids	0,289 \pm 0.084	1,335	2,838	0.031

618 **FIGURE CAPTIONS**

619 **Figure 1.** Probability that (A) a female *T. euproctidis* remains on a patch with 20 hosts and
620 (B) a female *A. ervi* remains on a patch with 60 hosts as a function of the time elapsed since it
621 entered the patch at three temperatures.

622

623 **Figure 2.** Graphical representation of the interaction between temperature and the effect of
624 each host rejection in *T. euproctidis*. Hazard ratios (\pm s.e.) are computed according to the
625 explanation provided by Wajnberg *et al.* (1999)

626

627 **Figure 3.** Proportion of time spent expressing each of the behaviors observed in *T.*
628 *euproctidis* females allowed to parasitize 20 *E. kuehniella* eggs at three temperatures.

629

630 **Figure 4.** Effect of temperature on the walking speed of *Trichogramma euproctidis* females

631

632 **Figure 5.** Mean number of kicking (A), jerking (B) and escaping (C) aphids attacked by the
633 parasitoid *A. ervi* at three temperatures. Mean \pm S.E. Different letters indicate significant
634 differences between temperature for each behavior.

635

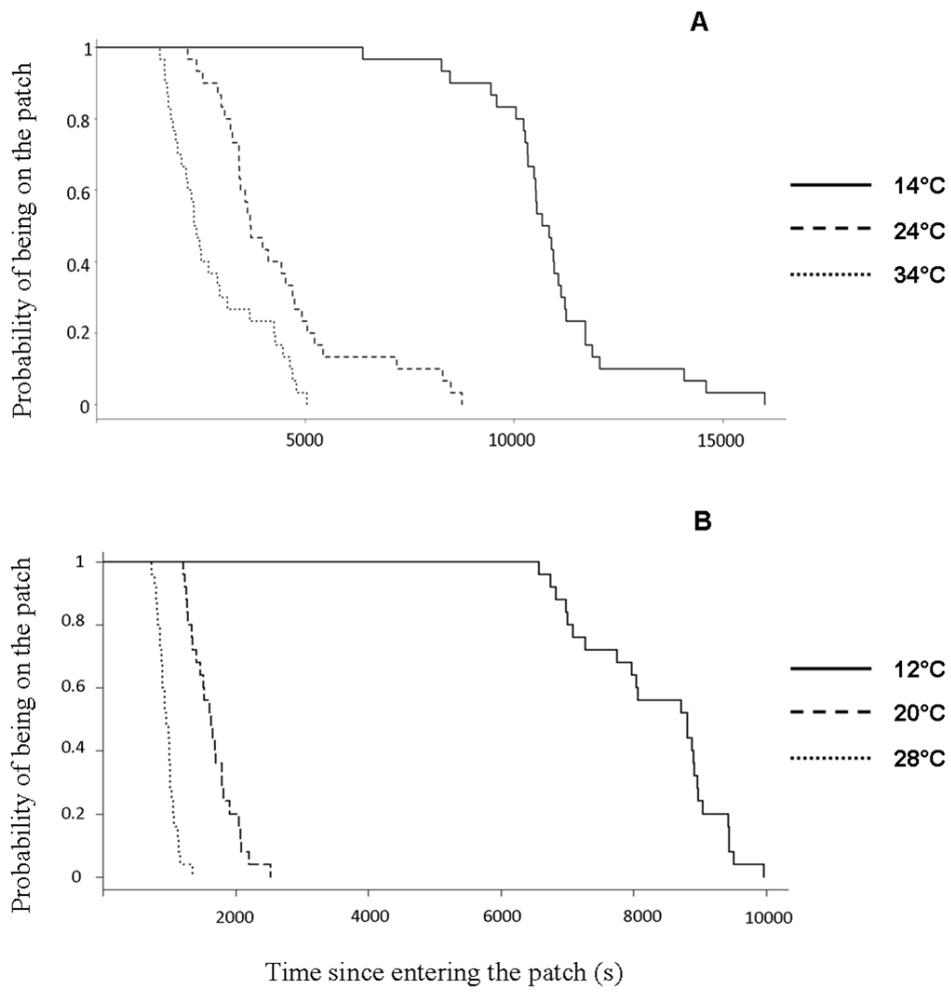


Figure 1

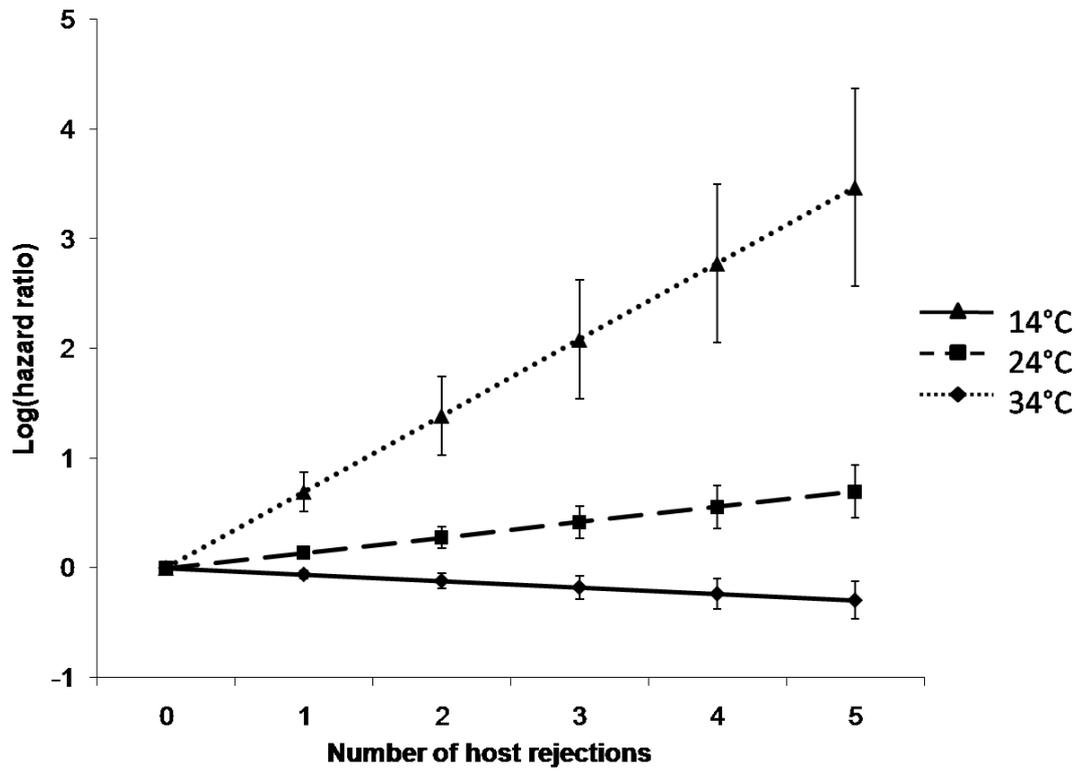
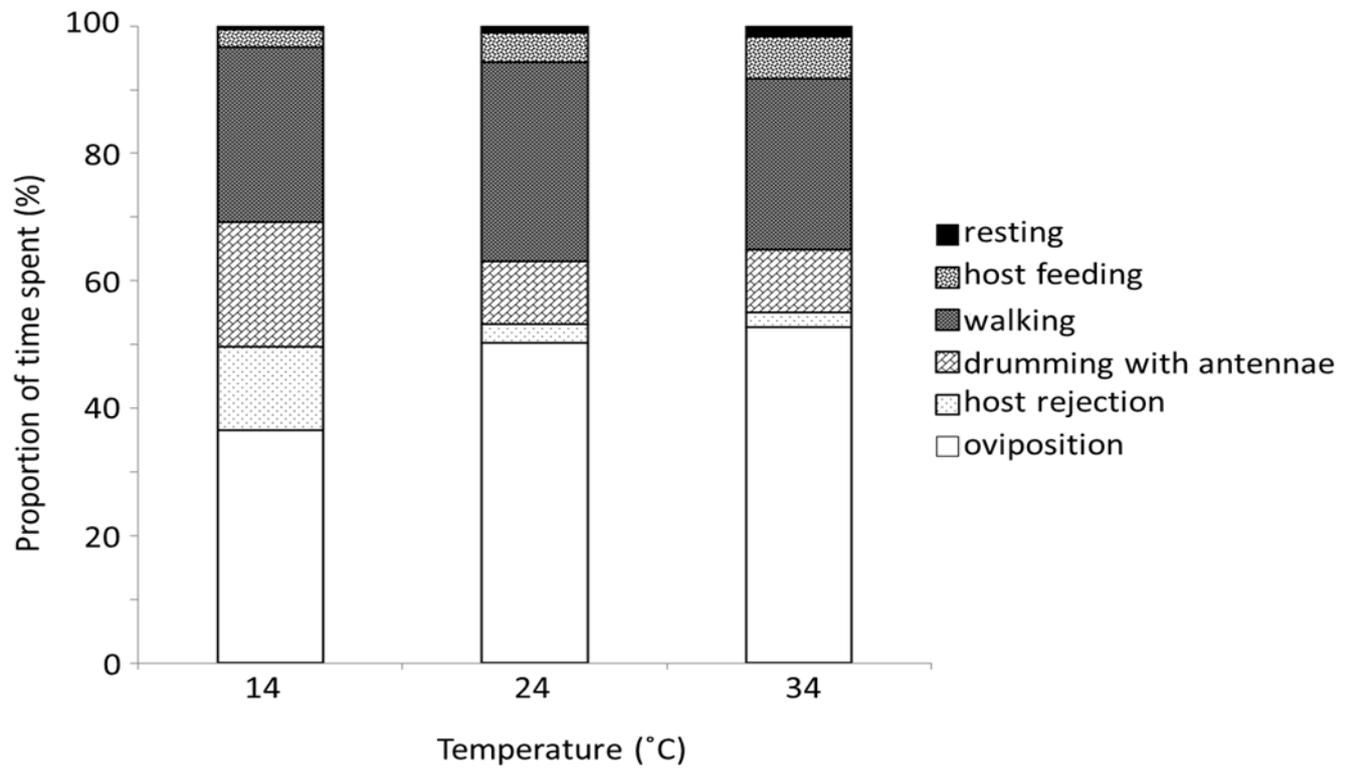


Figure 2

**Figure 3**

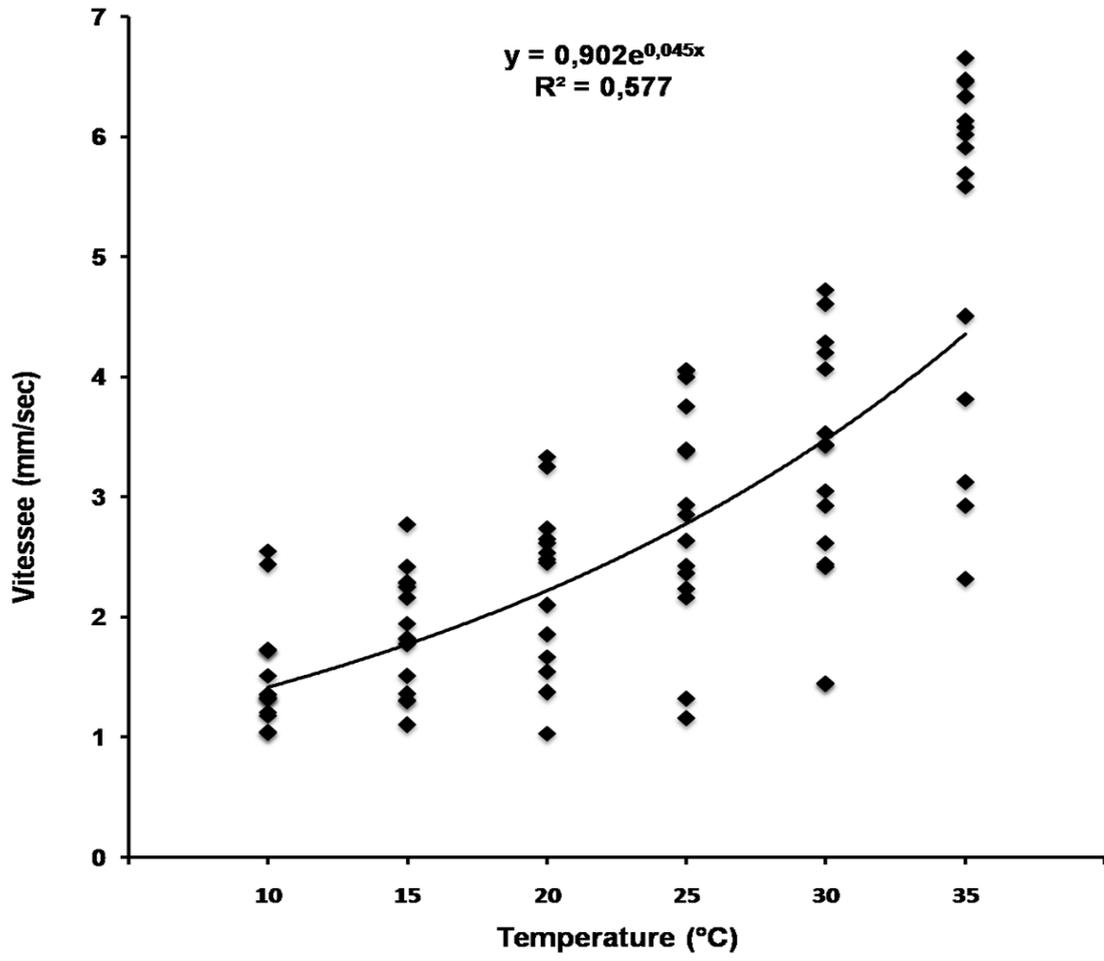


Figure 4

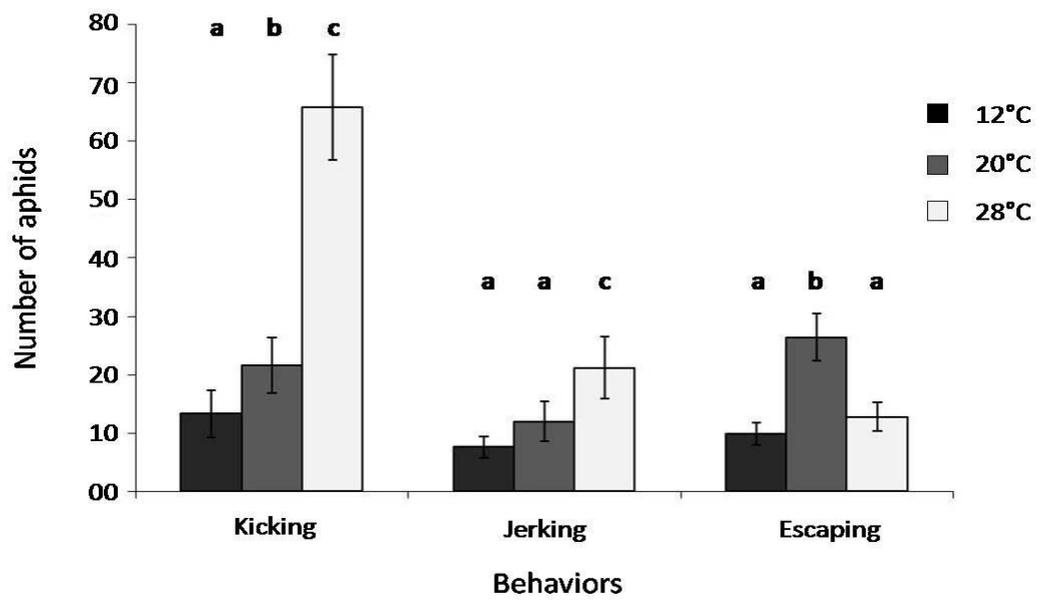


Figure 5