

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

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Abstract Patch time allocation has received much attention in the context of optimal foraging theory, including the effect of environmental variables. We investigated the direct role of temperature on patch time allocation by parasitoids through physiological and behavioural mechanisms and its indirect role via changes in sex allocation and behavioural defences of the hosts. We compared the influence of foraging temperature on patch residence time between an egg parasitoid, *Trichogramma euproctidis*, and an aphid parasitoid, *Aphidius ervi*. The latter attacks hosts that are able to actively defend themselves, and may thus indirectly influence patch time allocation of the parasitoid. Patch residence time decreased with an increase in temperature in both species. The increased activity levels with warming, as evidenced by the increase in walking speed, partially explained these variations, but other mechanisms were involved. In *T. euproctidis*, the ability to externally discriminate parasitised hosts decreased at low temperature, resulting in a longer patch residence time. Changes in sex allocation with temperature did not explain changes in patch time allocation in this species. For *A. ervi*,

we observed that aphids frequently escaped at intermediate temperature and defended themselves aggressively at high temperature, but displayed few defence mechanisms at low temperature. These defensive behaviours resulted in a decreased patch residence time for the parasitoid and partly explained the fact that *A. ervi* remained for a shorter time at the intermediate and high temperatures than at the lowest temperature. Our results suggest that global warming may affect host-parasitoid interactions through complex mechanisms including both direct and indirect effects on parasitoid patch time allocation.

Keywords Patch time allocation · Host-parasitoid interaction · Climate · Discrimination ability · Sex allocation · Anti-predator behaviours

Introduction

Resources such as food, mate or hosts are usually heterogeneously distributed in the environment and their exploitation requires time. One of the main decisions an organism faces when foraging is how to manage residence time between visited patches in order to maximise fitness yield (Wajnberg 2006). Patch time optimisation received much attention in the context of optimal foraging and was first addressed by Charnov (1976) in his marginal value theorem (MVT). The main assumption of the MVT is that a forager should leave a depleted patch when the current rate of gain falls below the average rate it would come across in the environment, thus maximising the long-term rate of fitness gain. This model is one of the most influential in behavioural ecology, but it has been subjected to criticism as it assumes that foragers are omniscient, and does not take into account proximate behavioural mechanisms involved in patch time allocation (van

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Alphen and Bernstein 2008) or how these mechanisms are affected by environmental constraints.

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

Despite the considerable influence of temperature on life history, physiological and behavioural traits of insects (Huey and Kingsolver 1989; Nylin and Gotthard 1998; Angilletta et al. 2004), its influence on patch time allocation has rarely been addressed. This issue is particularly important in the context of climate changes because increases in average temperature, temperature variance and frequency of extreme climatic events are expected to continue in the coming decades (IPCC 2007). Temperature may directly affect patch residence time through physiological or behavioural mechanisms. Firstly, walking speed, search rate, handling time and related behaviours are known to change with temperature in ectotherms because of its influence on physiological processes (see the Metabolic Theory of Ecology, Brown et al. 2004; Moiroux et al. 2012). Usually, walking speed and search rate follow a hump-shaped relationship with temperature in insects (Sentis et al. 2012). Since there is a link between walking, which is commonly used in parasitoids when foraging, and patch time allocation (Wajnberg et al. 2013), we may expect that patch residence time would vary with temperature as a direct consequence of its influence on the speed of movements. Secondly, behavioural mechanisms may have been selected in response to temperature. To our knowledge, only Amat et al. (2006) have directly addressed this issue in a parasitoid. They observed that a drop in temperature before foraging increased patch residence time and that this environmental cue was more important than foraging temperature itself. The authors proposed that thermal variations may be perceived as a signal of unfavourable weather conditions and that searching for a new patch would increase mortality risk or physiological costs. Finally, multi-objective behavioural mechanisms related to patch time allocation may be adopted by animals in response to temperature. Wajnberg (2012) clearly showed that sex allocation influenced patch time allocation in the egg parasitoid *Trichogramma chilonis* as laying a son did not change its patch residence time, while laying a

daughter decreased its patch time allocation. Considering that haplodiploid parasitoids can adjust sex allocation to their foraging temperature (Moiroux et al. 2014), we thus expect that females would also change their patch time allocation according to sex allocation, staying longer on a patch at higher temperatures because of the higher probability of laying a son.

Temperature may also indirectly influence patch time allocation via its effect on the nature and frequency of host defences (e.g. Miyatake et al. 2008; Ma and Ma 2012). For example, when exposed to alarm pheromone, pea aphids *Acyrtosiphon pisum* are less likely to drop from the feeding site to the ground when the environment is hot and dry than when it is more benign (Dill et al. 1990). More recently, Le Lann et al. (2014) showed that the total number of defence behaviours displayed by the grain aphid *Sitobion avenae* increased with a rise of temperature. Temperature may thus affect patch time allocation through its influence on host defences since parasitoids can change patch time allocation as a response to host defence behaviours. For example, the ichneumonid *Diadegma semiclausum* remains in patches longer when hosts drop off plants to defend against an attack, because the parasitoid waits for the host to return to the leaf (Wang and Keller 2003).

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

responses to temperature in two different biological systems, we expect our study to inform predictions of how host-parasite interactions will change in the context of ongoing global warming.

Materials and methods

T. euproctidis

Insect colonies

Trichogrammatidae wasps are small endoparasitoids of eggs of several lepidopteran species. The *T. euproctidis* strain used in this study originates from individuals sampled in Egypt and was maintained at 24 ± 1 °C, 50 % RH and LD 16:8 h on cold-killed eggs of *E. kuehniella*. Under these rearing conditions, the mean \pm standard error (s.e.) tibia length of this strain is 128 ± 9 μ m (J.-P. Parent, personal communication).

Patch residence time

Parasitised eggs were taken from the *T. euproctidis* colony and isolated in 300 μ L Beem[®] polyethylene capsules with a drop of honey. The next morning, newly emerged females were mated once with a virgin male, stored at 24 °C and transferred to a climate room at either 14, 24 or 34 °C 1 h before observation. The first and last temperatures are close to the species thermal limits but do not cause any deleterious effects on walking activity (see results on walking activity, Fig. 4). After this acclimatisation period, females were offered 20 cold-killed *E. kuehniella* eggs, placed side by side in 4 lines of 5 eggs on a filter paper disc. In this experiment, the patch was defined as the rectangular area formed by *E. kuehniella* eggs. The experimental arena was delimited by the plastic end piece of a camera ($\varnothing = 20$ mm) covered with Fluon to prevent females from climbing up the side. The number and duration of the following behaviours were recorded for 30 females per temperature with a camera (Dino-lite Pro AM413ZT, $\times 60$) and analysed using The Observer XT software (Noldus, Wageningen, The Netherlands): entering the patch, leaving the patch, walking, host feeding, antennation followed by oviposition, drilling, egg male deposition, egg female deposition, antennation followed by host rejection, host rejection and resting. Patch leaving was noted when females left the rectangle formed by *E. kuehniella* eggs and walked on the filter paper disc. Antennation followed by oviposition was recorded when a female exhibited non-linear movements on an egg, touched it repeatedly with its antennae and subsequently deposited an egg. Egg deposition was distinguished from drilling by positioning of the abdomen, which was perpendicular to the host when drilling and parallel during egg deposition. Egg female deposition was distinguished from egg male deposition by the

presence of a pause during oviposition sequence (Suzuki et al. 1984; Moiroux et al. 2014). Antennation followed by host rejection was recorded when a female exhibited non-linear movements on an egg, touched it repeatedly with its antennae and subsequently rejected a host. Host rejection consisted of drilling not followed by egg deposition. Walking was recorded when a female was moving, without recording pauses. Patch residence time was measured from the time the female entered the patch until she remained inactive for more than 30 min or walked off the patch for more than 3 min at 34 °C, 4 min 45 s at 24 °C and 7 min 30 s at 14 °C. These durations are proportional to average walking speed calculated at these three temperatures (i.e. 4.17 mm/s at 34 °C, 2.66 mm/s at 24 °C and 1.69 mm/s at 14 °C) using the equation of the regression of walking speed versus temperature, which was deduced from the experiment described below. Additional analyses were performed considering a similar threshold, i.e. females walked off the patch for more than 3 min for the three temperatures, and the results led to the same conclusion in both cases.

Walking speed

Newly emerged (<24 h), unfed females were placed individually in an empty Petri dish ($\varnothing 92$ mm) on a light table at 10, 15, 20, 25, 30 or 35 ± 1 °C. The movement of 15 females per temperature was video-recorded (Camera Panasonic WV-BD400) for 300 s and then analysed with a program developed by Vigneault et al. (1997). The contrast between individuals and the light table was used to track females and the program calculated the mean walking speed (mm/s), deduced from the distance travelled in 300 s minus duration of stops.

A. ervi

Insect colonies

A. ervi is a solitary koinobiont parasitoid of several aphid species. A few weeks prior to the tests, an *A. ervi* colony was established with individuals bought from BioBest Canada (Leamington, Canada). Parasitoids were reared in cages on the potato aphid *M. euphorbiae* at 20 ± 1 °C, 60 ± 10 % RH, 16 L:8 D photoperiod. In these rearing conditions, the mean \pm s.e. tibia length of this strain was 713 ± 5 μ m. The aphid colony was initiated with individuals collected from potato fields in the vicinity of Québec City, Canada. This colony was maintained on potato plants, *Solanum tuberosum* Linnaeus (Solanales: Solanaceae), cultivar “Norland” under the same conditions.

Patch residence time

Parasitised aphids were reared at 20 ± 1 °C on potato plants and isolated in gelatine capsules once they mummified. After emergence, each female parasitoid was kept with a male at 20

± 1 °C for 24 h in a Petri dish containing moistened cotton and honey. This period allowed females to mature eggs (Le Lann et al. 2010). The next day, their foraging behaviour was observed in climate chambers at 12, 20 or 28 °C. The first and last temperatures are close to *A. ervi* thermal extremes but do not induce any lethal effects or deleterious effects on walking activity (according to Gilchrist 1996). One hour before the observation, a potato leaf ($\phi \approx 4$ cm), which represented the patch in this experiment, was taped on a piece of paper and 60 apterous aphids were placed on it, 15 from each larval instar (L1 to L4). A range of different instars were exposed to *A. ervi* females because their defensive behaviours change with age (Barrette et al. 2010). Prior to the test, parasitoid females were acclimatised to the experimental temperature for 1 h. Females were then introduced in the experimental arena (ϕ 26 cm, h 8.5 cm) and the following behaviours were recorded with a camera (Panasonic WV-BD400) on 25 females per temperature and analysed with The Observer XT software (Noldus, Wageningen, The Netherlands): entering the patch, leaving the patch, walking, successful oviposition, failed oviposition, host rejection and resting. Patch leaving was noted when females walked or flew off the potato leaf. A successful oviposition was recorded when the parasitoid abdomen came into contact with the aphid while a failed oviposition was recorded when a parasitoid initiated an attack but failed to touch the aphid with its abdomen because of aphid behavioural defences. Host rejection was recorded when a female parasitoid came into contact with a motionless aphid and did not attack it. Both the number and duration of these behaviours were recorded. Patch residence time was measured from the time the female entered the patch until she left it for more than 1 min at 28 °C, 1 m 45 s at 20 °C and 7 min at 12 °C or stopped any activity for more than 15 min. These durations are proportional to average walking speed measured at these three temperatures by Gilchrist (1996): i.e. 17.9 mm/s at 28 °C, 10.7 mm/s at 20 °C and 2.6 mm/s at 12 °C. Additional analyses were performed considering a similar threshold, i.e. females left the patch for more than 1 min for the three temperatures, and the results led to the same conclusion in both cases.

During this test, we also recorded aphid anti-predator behaviours, i.e. kicking, body jerking or escaping from the patch. Kicking was recorded when an aphid extended one of its leg, usually a hind leg, and shook it up and down. Body jerking consisted of spasmodic horizontal movements of the abdomen. Escaping the patch was recorded when an aphid walked away after an attack and left the potato leaf. These behaviours were only recorded when aphids were attacked by the parasitoid. Cornicle secretions, which are common defences of aphids against natural enemies (Le Ralec et al. 2010), were not observed in our experiment.

For this parasitoid species, we were not able to study the influence of sex allocation on patch residence time. In *Aphidius* parasitoids, sex allocation cannot be determined by

observation. In his paper on multi-objective behavioural mechanisms, Wajnberg (2012) observed the sex of emerging parasitoids to assess sex allocation during oviposition. However, the temperature is known to affect sex ratio via physiological constraints on egg fertilisation in haplodiploid parasitoids (Moiroux et al. 2014), resulting in differences between sex allocation and realised sex ratio. Observation of the sex of emerging parasitoids was thus not reliable to evaluate sex allocation in our experiment.

Statistical analyses

Patch residence time was analysed using a Cox proportional hazard model (Cox 1972; Tenhumberg et al. 2001). Such model is classically used to analyse censored data such as patch residence time (Wajnberg et al. 1999). It determines a patch-leaving tendency for a foraging parasitoid, i.e. the probability per unit of time that a female leaves the patch, providing that she is still on it. It is formulated in terms of hazard ratio; a hazard ratio above 1 indicates an increase in the female's patch-leaving tendency and a hazard ratio below 1 indicates a decrease in the patch-leaving tendency. For *T. euproctidis*, temperature was considered as categorical factor, while the number of male ovipositions, number of female ovipositions, number of host rejections, number of host feeding events, resting periods and temporary exits from the patch were included as time-dependent covariates. The interaction between temperature and the number of ovipositions of sons and daughters, and the interaction between temperature and host rejections, were also considered as covariates in our models. For *A. ervi*, temperature (considered as a categorical factor), number of successful ovipositions, number of failed ovipositions, number of host rejections, number of aggressive behaviours (i.e. kicking + jerking) and of escaping aphids and number of temporary exits from the patch were included in the model, as were interactions between temperature and the number of successful or failed ovipositions. In both Cox models, the adequacy of the model was assessed by plotting deviance residuals (Therneau et al. 1990). The assumption that hazard rates were proportional for different values of covariates was also checked following Collett (1994) and Wajnberg et al. (1999).

In addition, the influence of temperature on the total number of ovipositions, host rejections, host feeding periods and resting periods for *T. euproctidis* was analysed using generalised linear models (GLM) with a Poisson error distribution for each dependent behavioural variable. After a GLM, multiple comparisons between temperatures were performed with Tukey multiple comparisons (performed in the scale of the link function), implemented with the "multcomp" package in R software. Data on sex allocation have been previously published in Moiroux et al. (2014). The influence of temperature on the proportion of time devoted to each of the

behaviours recorded was analysed with ANOVAs followed by Tukey tests after verifying that these data were normally distributed. For *A. ervi*, the influence of temperature on the number and duration of parasitoids behaviours is described in Moiroux et al. (2015). The number of kicking, jerking and escaping aphids was analysed using a GLM with a Poisson error distribution, considering temperature as a categorical factor and each of these behaviours as a dependent variable. Comparisons among temperatures were performed with Tukey multiple comparisons tests, as above. We fitted an exponential model to data of temperature versus *T. euproctidis* walking speed, because the range of temperatures tested was observed to lie in the exponential, increasing portion of this behaviour’s thermal performance curve (i.e. we saw no evidence of having bypassed the thermal optimum). All statistical analyses were carried out using R software version 2.14.1 (R Development Core Team 2013).

Results

T. euproctidis

Patch residence time

Temperature, the number of ovipositions of sons and daughters and the interaction between temperature and the number of hosts’ rejection significantly influenced the patch residence time of *T. euproctidis* (Table 1), while host rejection, host feeding, the number of temporary exits, the number of resting periods and the interaction between temperature and ovipositions of sons or daughters did not. The tendency to leave the patch increased with temperature (Fig. 1) and following a male and female oviposition (i.e. hazard ratio >1, Table 1). It should be noted that laying a son or a daughter increased patch

leaving tendency by very similar margins (respectively 0.558 ± 0.166 vs 0.576 ± 0.077). Host rejection increased the tendency to leave the patch at intermediate and high temperatures, but it decreased patch leaving tendency at low temperature (Fig. 2).

Temperature did not influence the total number of ovipositions, while the number of host rejections was significantly higher at 14 °C than at 24 or 34 °C, and females host-fed more at 34 °C (Table 2). Females foraging at 14 °C spent proportionally less time laying eggs ($F_{2, 87}=4.076, p=0.020$) than females tested at 24 and 34 °C, but spent proportionally more time performing antennation followed by host rejection ($F_{2, 87}=34.17, p<0.001$) and rejecting hosts ($F_{2, 87}=81.17, p<0.001$). Females foraging at 34 °C spent proportionally more time feeding on hosts than other females ($F_{2, 87}=13.61, p<0.001$), while females foraging at 20 °C spent proportionally more time walking than other females ($F_{2, 87}=4.601, p=0.020$). We did not observe any significant difference in the proportion of time spent resting ($F_{2, 87}=1.081, p=0.247$) or performing antennation followed by oviposition ($F_{2, 87}=0.808, p=0.318$) (Fig. 3).

Walking speed

Walking speed of *T. euproctidis* increased significantly with increasing temperature ($F_{1, 87}=118.7, p<0.001$; Fig. 4).

A. ervi

Patch residence time

Patch residence time was significantly influenced by temperature (Fig. 1b), the number of successful ovipositions, the number of escaping aphids and the number of aggressive aphids, but not by other time-dependent covariates or the

Table 1 Estimated regression coefficients (β) and their standard errors (s.e.), and hazard ratios (exp (β)) obtained for the covariates of the simplified Cox proportional hazard model relating patch residence time, temperature and observed behaviours in *T. euproctidis* (upper part); and

for the non-significant covariates of the initial Cox proportional hazard model relating patch residence time, temperature and observed behaviours (lower part). Parameter estimates are relative to a female which has not yet laid on a patch at 14 °C

	$\beta \pm \text{s.e.}$	exp (β)	χ^2 (df)	p value
Temperature (24 °C)	2.189 ± 0.765	8.933	22.778 (2)	<0.001
Temperature (34 °C)	2.654 ± 0.949	14.208		
Male oviposition	0.558 ± 0.166	1.747	11.359 (1)	<0.001
Female oviposition	0.576 ± 0.077	1.779	82.295 (1)	<0.001
Host rejection	-0.059 ± 0.035	0.942	0.005 (1)	0.941
Temperature (24 °C) × rejection	0.136 ± 0.062	1.146	18.198 (2)	<0.001
Temperature (34 °C) × rejection	0.754 ± 0.184	2.126		
Host feeding	0.089 ± 0.102	1.093	2.091 (1)	0.382
Resting periods	-0.094 ± 0.162	0.910	1.408 (1)	0.563
Temporary exits	0.103 ± 0.005	1.108	1.374 (1)	0.523

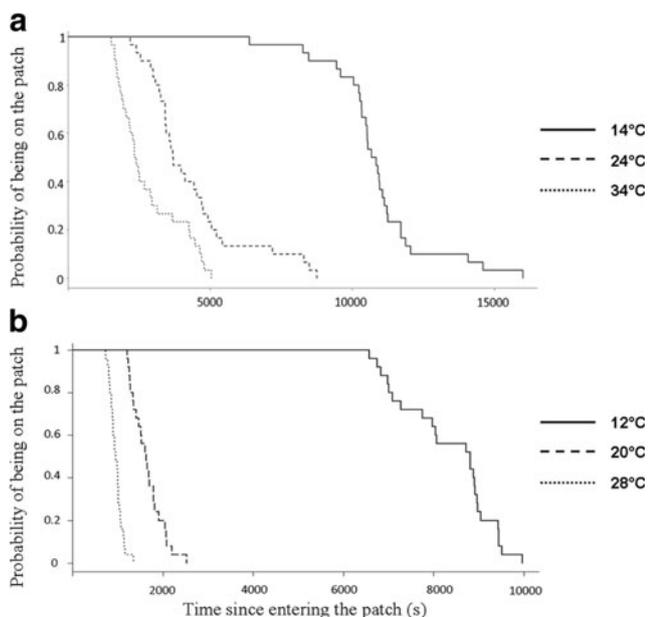


Fig. 1 Probability that **a** a female *T. euproctidis* remains on a patch with 20 hosts and **b** a female *A. ervi* remains on a patch with 60 hosts as a function of the time elapsed since it entered the patch at three temperatures

interactions between temperature and any of the time-dependent covariates (Table 3). Females remained longer in the patch following ovipositions and left earlier with increasing temperature and the number of aggressive or escaping aphids.

Aphids

The number of kicks performed by *M. euphorbiae* (GLM with Poisson errors, $df=1$, $\chi^2=19.758$, $p<0.001$) and the number of aphids jerking (GLM with Poisson errors, $df=1$, $\chi^2=8.667$, $p=0.002$) were significantly influenced by the

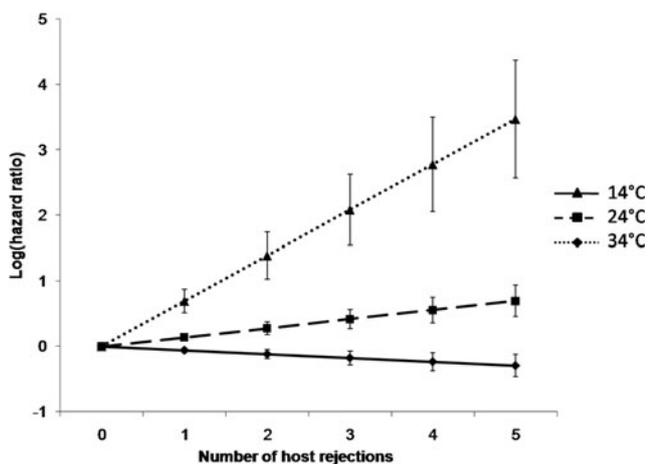


Fig. 2 Graphical representation of the interaction between temperature and the effect of each host rejection in *T. euproctidis*. Hazard ratios (\pm s.e.) are computed according to the explanation provided by Wajnberg et al. (1999)

foraging temperature. Aggressive defensive behaviours were more frequent at 28 °C and less frequent at 12 °C (Fig. 5). Foraging temperature significantly influenced the number of aphids escaping from the patch when attacked (GLM with Poisson errors, $df=1$, $\chi^2=14.504$, $p<0.001$). Escape behaviour was the most frequent in aphids attacked at 20 °C (Fig. 5).

Discussion

We investigated the influence of temperature on patch time allocation in two parasitoid species, considering its direct effects through physiological or behavioural mechanisms and its indirect effects via changes in sex allocation and behavioural defences of hosts. Our main results are that (1) patch residence time decreased as temperature increased, mainly because of an increase in walking speed for both *T. euproctidis* and *A. ervi*; and (2) additional mechanisms explained changes in patch residence time with temperature, but these mechanisms were different between the two species (a decrease in external discrimination ability at low temperature for *T. euproctidis*, and thermal variations in nature and frequency of host defensive behaviours for *A. ervi*). We believe that this is the most comprehensive evidence collected to date showing that foraging temperature interacts with the proximate behavioural mechanisms underlying parasitoid patch time allocation.

Patch time allocation

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

On the contrary, successful ovipositions were associated with increased patch residence time in *A. ervi*. This incremental mechanism (Waage 1979) has been observed in many parasitoid species (for a review, see Wajnberg 2006) including a close and sympatric species, *Aphidius rhopalosiphii* (Outreman et al. 2005). Such behaviour is expected when organisms face a large variance in patch quality (Iwasa et al. 1981), as observed for aphid colonies in the field (e.g. Ward et al. 1986).

Patch time allocation and temperature

In both parasitoid species, patch residence time decreased sharply as temperature increased. This result contrasts with a

Table 2 Total number of ovipositions, host rejections, host feeding and resting periods measured on *T. euproctidis* females on patches of 20 cold-killed *E. kuehniella* eggs at three temperatures. Host rejection refers to a

drilling not followed by egg deposition. Mean ± SE. Model outputs are from GLMs (Poisson error distribution) with temperature as a categorical factor and each behaviour as dependent variable

Temperature (°C)	Oviposition	Host rejection	Host feeding	Resting period
14	18.60 ± 0.23	15.27 ± 1.02	1.62 ± 0.25	0.80 ± 0.19
24	18.63 ± 0.33	3.45 ± 0.14	1.83 ± 0.23	0.40 ± 0.15
34	18.57 ± 0.33	2.17 ± 0.4	2.77 ± 0.50	0.53 ± 0.19
χ^2 (df=1)	0.001	40.402	9.331	1.854
p value	0.976	<0.001	0.002	0.173

study by van Roermund et al. (1994) who did not observe any influence of temperature on patch time allocation in the whitefly parasitoid *Encarsia formosa*; however, in this previous study, parasitoids were tested on patches without hosts. These unnatural conditions likely explain the absence of parasitoid response to temperature.

An increase in female parasitoid activity with warming, as evidenced by the increase in walking speed in *T. euproctidis* (this study) and *A. ervi* (Gilchrist 1996), would probably modulate patch residence time. The higher the temperature, the higher the metabolic rate and the faster females perform the component behaviours that make up foraging up to an optimum, as predicted in the context of the Metabolic Theory of Ecology (Brown et al. 2004). In *T. euproctidis* and *A. ervi* (Gilchrist 1996), walking speed increased on average 1.7 times between 24 and 34 °C and 20 and 28 °C, respectively, while patch residence time and duration of the different behaviours recorded (antennation, drilling, egg deposition) decreased 1.7 times. This result suggests a simple physiological change associated with temperature. However, while the walking speed increased 1.6 times from 14 to 24 °C and 2.5

times from 14 to 34 °C in *T. euproctidis*, patch residence time decreased 2.6 and 8.7 times, respectively. In *A. ervi*, walking speed increased 4.1 times from 12 to 20 °C and 6.9 times from 12 to 28 °C (Gilchrist 1996), while patch residence time decreased 5 and 8.6 times, respectively. These differences suggest that in both species walking speed alone does not explain the increase in patch time allocation at the lowest temperature. From our results, different mechanisms may be involved for each species: a decrease in external discrimination ability in *T. euproctidis* and variations in nature and frequency of host defensive behaviours in *A. ervi*. These mechanisms were observed in parasitoids and hosts taken from the lab, but field populations living under changing environment may respond differently than these strains.

At the lowest temperature, the number of internal host rejections (i.e. a drilling not followed by egg deposition) by *T. euproctidis* females and the proportion of time spent performing antennation followed by an internal host rejection were higher than at other temperatures. Parasitised eggs were thus mainly rejected after perforation of the chorion by the ovipositor, but females were apparently unable to reject them

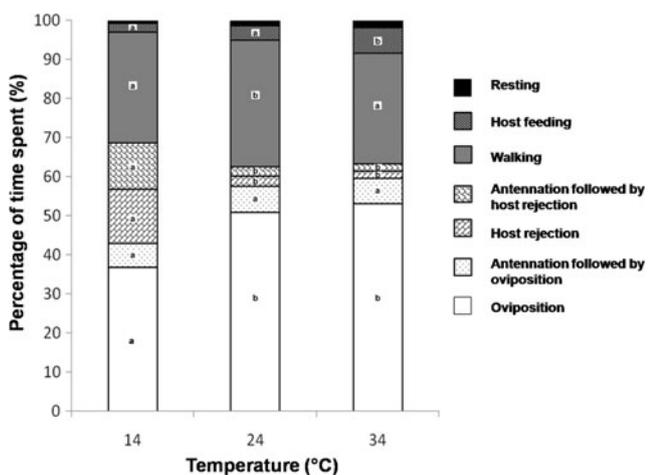


Fig. 3 Percentage of time spent expressing each of the behaviours observed in *T. euproctidis* females allowed to parasitise 20 *E. kuehniella* eggs at three temperatures. Different letters indicate significant differences between temperatures for each behaviour. There was no significant difference among temperatures for the proportion of time spent resting

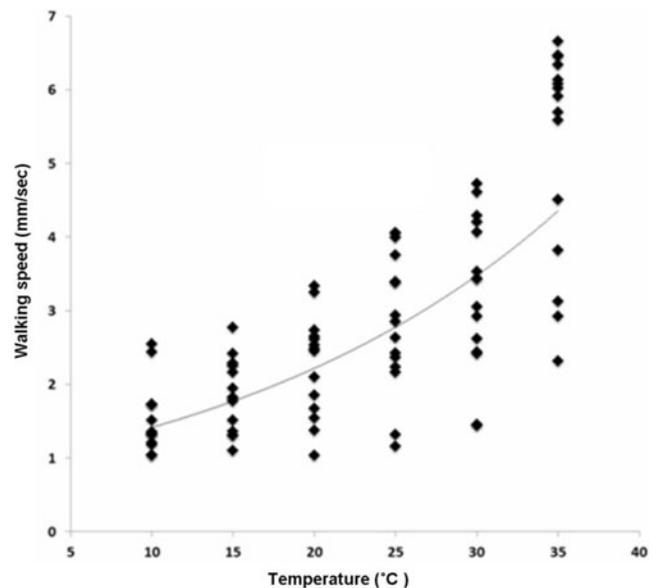


Fig. 4 Effect of temperature on the walking speed of *Trichogramma euproctidis* females. Regression model: $y = 0.9022e^{0.045x}$, $R^2 = 0.577$

Table 3 Estimated regression coefficients (β) and their standard errors (s.e.), and hazard ratios (exp (β)) for the significant covariates of the simplified Cox proportional hazard model relating patch residence time, temperature and behaviours of *A. ervi* females allowed to attack four stages of *M. euphorbiae* (upper part); and for the non-significant covariates the initial Cox proportional hazard model relating patch residence time, temperature and observed behaviours (lower part). Parameter estimates are relative to a female which has not yet laid on a patch at 12 °C

	$\beta \pm \text{s.e.}$	exp (β)	χ^2 (df)	p value
Temperature (20 °C)	1.817 \pm 0.528	6.153	20.337 (2)	<0.001
Temperature (28 °C)	2.344 \pm 0.712	10.423		
Successful oviposition	-0.348 \pm 0.104	0.706	17.877 (1)	0.035
Aggressive aphids	0.333 \pm 0.092	1.395	38.451 (1)	0.016
Escaping aphids	0.289 \pm 0.084	1.335	24.744 (1)	0.031
Host rejection	-0.110 \pm 0.096	0.896	1.323 (1)	0.408
Failed oviposition	0.018 \pm 0.012	1.018	1.639 (1)	0.353
Temporary exits	-0.004 \pm 0.006	0.009	0.014 (1)	0.671

while drumming with their antennae. At 24 and 34 °C, females very rarely stopped to perform antennation on already-parasitised hosts; this behaviour may thus have been underestimated at these two temperatures since it was recorded as walking. However, at 14 °C, females systematically displayed a complete antennation on already-parasitised hosts, explaining the strong increase in the proportion of time spent performing antennation followed by host rejection. These results indicate that a low temperature during oviposition affected the external discrimination ability of *T. euproctidis* females, which probably contributed to the increased patch residence time recorded at 14 °C. Overall, the same number of eggs was parasitised by females across tested temperatures, but it took more time to exploit the patch at low temperature. Several non-exclusive hypotheses can explain the decrease in external discrimination ability. First, the amount or composition of the

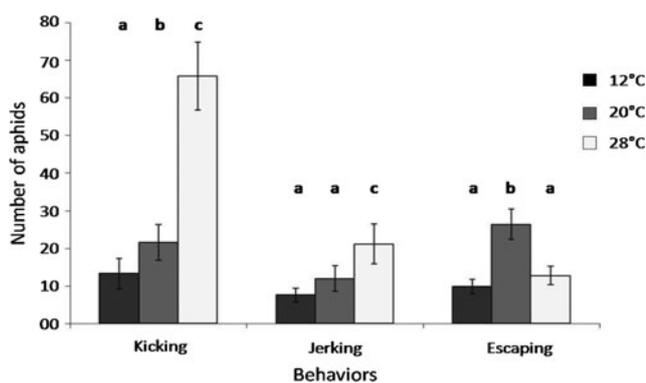


Fig. 5 Mean number of kicking (a), jerking (b) and escaping (c) aphids attacked by the parasitoid *A. ervi* at three temperatures. Mean \pm s.e. Different letters indicate significant differences between temperatures for each behaviour (Tukey multiple comparisons, $p < 0.05$)

marking pheromone produced by a female and left on the host after oviposition (Roitberg and Mangel 1988) may be changed at low temperature, as observed for the marking pheromone produced by beewolf males, *Philanthus triangulum*, to mark their territories (Roeser-Mueller et al. 2010). Secondly, the receptivity of antennae to chemical signals or their integration in the olfactory lobe may also be affected, as suggested for European corn borer males, *Ostrinia nubilalis*, exposed to female sex pheromone at different temperatures (Hilker and McNeil 2008). An effect of low temperature on the nervous system, especially on learning ability, was also proposed by van Baaren et al. (2005) to account for the absence of external discrimination ability in cold-stored females of the myrmard *Anaphes victus*.

This negative effect of low temperature on external discrimination ability probably explains that we observed a significant influence of the interaction between temperature and host rejection on patch residence time in *T. euproctidis*. At intermediate and high temperatures, *Trichogramma* females left earlier the patch after host rejection. This decremental mechanism is assumed to be adaptive and has been commonly reported in parasitoids (Wajnberg 2006) including species from the *Trichogramma* genus (Wajnberg et al. 2003). At low temperature, we observed an incremental mechanism associated with host rejection. This behavioural mechanism has only been reported in one parasitoid species (Nelson and Roitberg 1995) and is probably the consequence of deleterious effect of low temperature on sensory structures in our experiment. *A. ervi* did not suffer as much as *T. euproctidis* from low temperature. It is likely that interspecific variations or local adaptations of reproductive behaviours at low temperature exist since oviposition does not seem to be negatively affected by temperatures close to 0 °C in some parasitoids (Meiners et al. 2006). Size differences between the species might for example explain these differences. Due to their small size and thermoregulatory constrains, egg parasitoids might be physiologically more affected by changes in temperature than the larger aphid parasitoids.

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

In *A. ervi*, the nature and frequency of defensive behaviours displayed by aphids changed with temperature. They frequently defended themselves by kicking and jerking at the highest temperature while they tended to escape at the intermediate temperature. This observation is consistent with the results on pea aphids, *Ac. pisum*, which are less likely to drop from their feeding site when the environment is hot and dry than when it is more benign (Dill et al. 1990). Aphids may be less prone to leave a leaf when temperature is high as they need more resources to compensate for the increased metabolic rate (Brown et al. 2004) and should actively defend against parasitoids instead of escaping. Aphid aggressive behaviours resulted in an increase in patch leaving tendency for the parasitoid *A. ervi*. This effect may explain why the patch residence time of aphid parasitoids was shorter at 28 °C than expected from the change in walking speed when compared to the lowest temperature, at which aphids rarely defended. Since aggressive host behavioural defences increase injury risk for parasitoids, it is likely that the optimal behaviour would be to search for another patch with less aggressive hosts. To our knowledge, this is the first experimental evidence that such aggressive behavioural defences influence the behavioural mechanisms underlying patch time allocation in insect natural enemies. We also observed that patch residence time decreased with the number of aphids leaving the patch when exposed to a parasitoid. This last result contrasts with the observation on the ichneumonid parasitoid *D. semiclausum* that remained longer on a patch when hosts temporally left after attack (Wang and Keller 2003), waiting for the discovered host to climb back to the leaf (Wang and Keller 2002). *A. ervi* instead left the patch earlier, probably because females perceived a decrease in patch quality (e.g. Boivin et al. 2004; Outreman et al. 2001). This result may explain why *A. ervi* remained shorter than expected at the intermediate temperature compared to the lowest temperature, since aphids escaped more frequently at intermediate temperature.

Conclusion

Patch time allocation is influenced by several environmental variables, including temperature. We present evidence that foraging temperatures directly influence patch time allocation in parasitoids, mainly through physiological mechanisms, and indirectly through changes in the nature and frequency of host behavioural defences. In the next decades, average temperature, temperature variance and frequency of extreme climatic events are expected to increase (IPCC 2007). From our results, we might predict that the behaviour of some species will be more affected than others, as we observed a significant effect of the interaction between temperature and host rejections on patch leaving tendency in *T. euproctidis* but not in *A. ervi*. In parasitoid species attacking hosts that actively defend

themselves, the influence of the predicted climate change on patch time allocation might be more closely related to the induced changes in host behavioural defences than the direct effect of temperature on the parasitoid itself. This result suggests that both parasitoids and hosts should be considered when investigating the role of climate on patch residence time.

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