Original Article

Thermal stress affects patch time allocation by preventing forgetting in a parasitoid wasp

Paul K. Abram,[a,b] Antonino Cusumano,[c] Ezio Peri,[c] Jacques Brodeur,[a] Guy Boivin,[b] and Stefano Colazza[c]

[a]Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, H1X 2B2, Montréal, Québec, Canada, [b]Centre de Recherche et de Développement en Horticulture, Agriculture et Agroalimentaire Canada, Saint-Jean-sur-Richelieu, Canada, and [c]Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Italy

Learning and memory allow animals to adjust their foraging strategies through experience. Despite the known impact of temperature on many aspects of the behavioral ecology of animals, memory retention in the face of realistic thermal stress has seldom been assessed. In the laboratory, we studied the behavioral expression of an egg parasitoid's (*Trissolcus basalis*) memory when exposed to thermal stress that could be encountered in nature. We hypothesized that thermal stress would disrupt memory consolidation and/or modify the optimality of memory retention, thus affecting patch time allocation strategies. Memory consolidation was resilient to 1 h of thermal stress following an unrewarded experience (learning) on a patch of host-associated infochemicals. Neither high (40 °C) or low (10 °C) thermal stress changed the intensity of the experienced wasps' behavioral response relative to those held at a moderate temperature (25 °C). Next, we investigated how temperature stress could affect the parasitoids' memory retention ("forgetting"). When kept at a constant moderate temperature after learning, residence times of wasps retested on host cues increased relative to controls (naïve wasps) over a period of 4 days as they presumably "forgot." However, both hot and cool daily temperature cycles prevented forgetting; the residence times of retested experienced wasps in these treatments did not change relative to controls over time. We discuss to what extent this may be an adaptive response by the parasitoids versus a physiological constraint imposed by temperature. Our findings contribute to an understanding of the impact of thermal stress on foraging strategies that involve learning and memory.

Key Words: **foraging, host associated cues, learning, optimal memory window, temperature, *Trissolcus basalis*.**

INTRODUCTION

Learning and memory allow organisms to use information from past experiences to adjust their behavior. Memories of learned experiences should not be retained indefinitely, however—as individuals move through their environment in space and time, information regarding the past may eventually become unreliable and it may be adaptive to "forget" after a certain amount of time (Kraemer and Golding 1997). This optimal memory window is thought to be dynamic within individuals, depending on factors such as the direct costs of memory retention, environmental variability, and individual state (e.g., age, energy level; Dunlap et al. 2009). An unexplored aspect of this framework is that animals could adaptively adjust the length of memory retention in response to abiotic stressors in the environment. Alternatively, abiotic environmental stress could act as a constraint by interfering with the mechanisms underlying memory consolidation or retention (Beck

and Rankin 1995; Sangha et al. 2003; Knezevic et al. 2011; Teskey et al. 2012). However, the effect of natural abiotic stress on memory retention remains poorly understood, particularly in an ecological context.

Temperature is one of the most important abiotic factors affecting the ecology of many organisms. For ectotherm animals, which cannot typically regulate their body temperature internally, the rate of metabolic processes is in large part determined by environmental temperature (Gillooly et al. 2001; Clarke 2003), which scales up to affect many aspects of their behavior and ecology (Brown et al. 2004). For a given population, the functioning of physiological and behavioral processes is typically optimal within a certain thermal range. At "extreme" or "stressful" temperatures (i.e., near the lower and upper limits of a given animal's thermal tolerance), performance tends to decrease (Huey and Kingsolver 1989; Angilletta et al. 2002). Because temperature fluctuations are becoming more frequent and extreme as a result of global climate change (Stocker et al. 2013), it is increasingly relevant to study the responses of ectotherm animals to extreme temperatures. Although some studies

have examined the effect of temperature stress during immature stages on subsequent adult learning capacity (e.g., van Baaren et al. 2005; Jones et al. 2005), studies on the effects of realistic temperature stress on memory retention following learned experiences are rare, and have involved simple behavioral responses (siphon withdrawl reflex of a gastropod; Sangha et al. 2003; Teskey et al. 2012) whose general ecological significance is difficult to ascertain.

Animal memory could respond to stressful temperatures in adaptive and/or nonadaptive ways. On one hand, exposure to stressful temperatures could act as a constraint by eliminating less stable forms of memory that are present soon after learning (while memory is being consolidated), as has been observed in many ectotherm species when applying low-temperature anaesthesia (e.g., Erber 1976; Xia et al. 1998; Sangha et al. 2003; van den Berg et al. 2011). However, the temperatures applied in past studies are typically more extreme than those that would be experienced by the study organisms in natural situations. Once memories are consolidated, temperature stress could alter the rate of active forgetting processes (Berry et al. 2012; Hadziselimovic et al. 2014). A further possibility is that animals could adaptively alter the length of memory retention in response to thermal stress. The model of Dunlap et al. (2009), supported by some empirical investigations (Pravosudov and Clayton 2001; Friedrich et al. 2004; Orsini et al. 2004), predicts that organisms in poor state (e.g., poor nutritional condition) with a high energetic cost of living should have the longest possible memory window, because employing a learned response to a familiar stimulus maximizes payoffs under these circumstances. It follows that the optimal memory window should increase under stressful thermal regimes when metabolic costs (e.g., lipid and carbohydrate consumption, cost of metabolic adaptations to thermal stress) are high. In order to examine the validity of these ideas, it is most relevant to test temperature regimes that occur in nature, in biological systems where the behaviors related to memories are closely linked to fitness.

Parasitoids are insects whose eggs and larvae develop in or on the body of other arthropods, whereas the adults are free-living (Quicke 1997). These organisms face the problem of finding hosts that are distributed in patches throughout complex and heterogenous environments. In order to focus foraging effort in areas where hosts are most likely to be present, many species of parasitoids make use of infochemical cues that may be associated with, but do not guarantee, the presence of hosts suitable for parasitism (i.e., indirect host-associated cues) (Fatouros et al. 2008). Learning allows foraging parasitoids to dynamically adjust their sensitivity to these infochemicals based on how reliably they signal the presence of hosts, and adjust their patch time allocation strategies accordingly (Vet 1999; Fatouros et al. 2008; Vinson 1998).

Trissolcus basalis (Wollaston) (Hymenoptera: Platygastridae), a parasitoid of stink bug (Hemiptera: Pentatomidae) eggs, uses host adult walking traces as an indirect host-associated cue (Colazza et al. 1999). When parasitoids encounter a substrate contaminated with host walking traces, they show an arrestment response characterized by an increased turning rate and reduced walking speed that focuses searching and thus increases their residence time in the kairomone-contaminated area (Colazza et al. 1999, 2014). When parasitoids are not rewarded with an oviposition in host eggs within a certain amount of time, their sensitivity to host walking traces progressively decreases, they eventually leave the contaminated area, and they show a less intense arrestment response (i.e., decreased residence time, faster walking speed, and lower turning tendency) on the next patch they encounter (Peri et al. 2006). However, the arrestment response of wasps with an unrewarded experience can be restored to levels typical of naïve wasps if the host footprints are associated with an oviposition in host eggs, or if more than 72 h elapses and they presumably "forget" (Peri et al. 2006). This foraging strategy, which depends on learning and memory, shows similarities to classic mechanistic models of parasitoid patch time allocation (Waage 1979; Wajnberg 2006) and the underlying processes may generalize across many taxa.

In this study, we tested the effect of short- and long-term temperature stress on the behavioral expression of memory (patch time allocation) in T. basalis following an unrewarded experience with indirect host-associated cues, under laboratory conditions. Following the above reasoning, we hypothesized that 1) realistic high and low temperature stress applied during memory consolidation would cause memory loss (amnesia), and 2) memory window (the length of memory retention) would be extended when wasps are held at stressful temperatures over the period of several days. This is the first study of the response of insect memory to thermal stress within a range that could be realistically experienced in nature, and should set the stage for future investigations that examine underlying mechanisms and potential ecological consequences of such stress.

METHODS

Study system

Trissolcus basalis is a minute (~1.5 mm in length), cosmopolitan egg parasitoid of many different species of hemipteran insects worldwide (Loch 2000; Salerno 2000; HOL 2015). One of its most closely associated host species, Nezara viridula (L.) (Hemiptera: Pentatomidae), is extremely polyphagous, having been reported on more than 30 families of host plants and is an important pest of soybean cultivations (Todd 1989; McPherson and McPherson 2000). Adult N. viridula often move between habitats during the same season, sometimes using different sites for feeding, mating, and oviposition (Todd 1989). Trissolcus basalis can use several different host-associated infochemical cues to localize host egg masses, including host-derived volatiles, herbivory- and oviposition-induced plant volatiles, and adult walking traces (reviewed in Fatouros et al. 2008; Conti and Colazza 2012; Colazza et al. 2014).

Insect colonies

Trissolcus basalis and N. viridula colonies were established from individuals collected in Western Sicily, Italy, in the summer of 2014. Trissolcus basalis colonies were maintained by exposing N. viridula egg masses to groups of 5–7 female wasps in cylindrical glass tubes (volume: 85 mL), and emerging offspring were fed with drops of pure liquid honey. Nezara viridula colonies were housed in ventilated wooden cages (47.5 cm × 34.5 cm × 34.5 cm) and fed with cabbage (Brassica oleracea L.) leaves, sunflower seeds, tomatoes, and bouquets of field-collected Solanum nigrum L. White paper towel was provided as an oviposition substrate, and egg masses were collected every 2–3 days to maintain the T. basalis and N. viridula colonies. Female T. basalis, assumed mated, were isolated from the colony and placed in a small PCR tube (0.2 mL) with a drop of pure liquid honey the day before they were used for experiments. Females were 2–6 days old when tested, and had no oviposition experience. All insect colonies were maintained at 26 ± 1 °C, 16:8 h light:dark, and 60 ± 10% relative humidity.

General bioassay protocol

To measure the intensity of the behavioral response of parasitoid females to host walking traces, we used a protocol similar to that of Peri et al. (2006). Briefly, bioassays were conducted in open arenas with a 25 cm × 25 cm sheet of filter paper, with a circular area (6-cm diameter) in the middle of the filter paper exposed to a single female *N. viridula* for 30 min, in order to treat it with the stink bug's walking traces (the rest of the arena was left uncontaminated). *Trissolcus basalis* females were then released singly into the center of the arena and observed until the wasp left the open arena (training bouts typically last about 200–250 s for naïve wasps; see Peri et al. 2006). The intensity of arrestment responses of parasitoids in these arenas in the various experimental treatments was assessed with "Xbug," an unpublished video tracking system and motion analysis software package, which allowed the determination of the parasitoid's patch residence time, mean linear velocity (walking speed), and tortuosity index (a measurement of how much the parasitoid's path deviated from a straight line; Peri et al. 2006). All bioassays took place in an isolated room maintained at 25 ± 1 °C, with lighting on the arena provided by two 19-cm-long fluorescent tubes.

For both experiments described below, parasitoids were exposed to temperature treatments by placing the closed PCR tubes containing the wasp in the heating block of a thermocycler (MasterCycler Personal, Eppendorf, Germany). To ensure that parasitoids were exposed to the temperature at which the thermocycler was set, they were confined to the part of the tube in contact with the heating block with a small piece of cotton inserted in the upper portion of the tube.

Experiment 1: the effect of thermal stress on memory consolidation

The goal of the first experiment was to test whether high or low temperature stress applied after an experience on host kairomones disrupted the parasitoids' memory consolidation. Although we do not know how long the anaesthesia-sensitive memory (ASM) phase lasts for *T. basalis*, this phase, during which memory is sensitive to cold shock, can range from less than an hour to more than 2 h in other parasitoids (van den Berg et al. 2011; Kruidhof et al. 2012; Schurmann et al. 2015) and is typically less than 1 h in *Drosophila* (Margulies et al. 2005). We thus chose to apply the temperature stress immediately after learning to maximize the chances of disrupting memory consolidation. The 3 temperature treatments were: 10 °C (low temperature stress), 25 °C (control), and 40 °C (high temperature stress). The 2 extreme temperatures are near the limits of the air temperature experienced by *T. basalis* during the period in which it has been recorded parasitizing hosts (May–October; Peri et al. 2014) under field conditions in Western Sicily. They are also near the upper (~42–43 °C) and lower (~8–9 °C) temperature thresholds that induce heat stupor and anaesthesia, respectively (Abram PK, unpublished data). 'Experienced' female parasitoids were obtained using the following procedure: wasps were 1) released on a first arena with host cues (hereafter, training), 2) recollected into a PCR tube once they left the arena (end of training), and exposed to 1 of the 3 temperature treatments for 1 h, 3) acclimated inside the same tube in the bioassay room at 25 °C for 15 min, and then 4) released and filmed on a second patch of host cues ("testing") at 25 °C, for a total training-testing interval of 1.25 h.

To obtain an estimate of wasps' baseline response to host cues in the absence of experience, and to disentangle the other effects of temperature stress from its effect on memory formation, we also tested "naïve" females that were exposed to the same temperature treatments and acclimation period but had no previous experience (no training). Replicates were eliminated if the females did not show an arrestment response (i.e., flew away immediately when placed on the patch) on the second patch of host cues (7.6% of trials; nonresponders were present in all treatments).

For each of the 6 treatments (naïve/experienced x 10 °C/25 °C/40 °C), between 38 and 46 successful replicates were performed, for a total of 239 wasps tested. Experiments were performed over a period of 9 days, between 8:45 and 15:30 h, with temperature and experience treatments balanced with respect to time of day.

Experiment 2: the effect of thermal stress on memory retention

The second experiment tested whether temperature affected the parasitoids' memory retention over a period of several days. Temperature data were obtained for Contessa Entellina, Italy (one of the collection sites for our culture of *T. basalis*), for 2009–2013. Because *T. basalis* actively forages for hosts between May and October in nature (Peri et al. 2014), we considered temperature data from only these months. The 10 days with the highest (all during July and August) and lowest (all during May or October) average temperatures were selected, and the hourly temperatures for each period were averaged to produce a thermal profile of "typical" cool and hot days that parasitoids could experience while foraging in nature (Figure 1). Experimental temperature regimes that could be reproduced in a thermocycler were visually fitted to each of these curves, having approximately the same average daily temperature (hot: ~30 °C, cool: ~16 °C; Figure 1). As a positive control, recreating the conditions of Peri et al. (2006) under which experience was forgotten after 72 h, we included a third temperature treatment where wasps were kept at a constant temperature of 25 ± 1 °C. Experiments were performed in blocks that included 1 of the 2 extreme temperature treatments and the control treatment; all blocks were then pooled for analysis after testing for similarity of the control treatment across blocks.
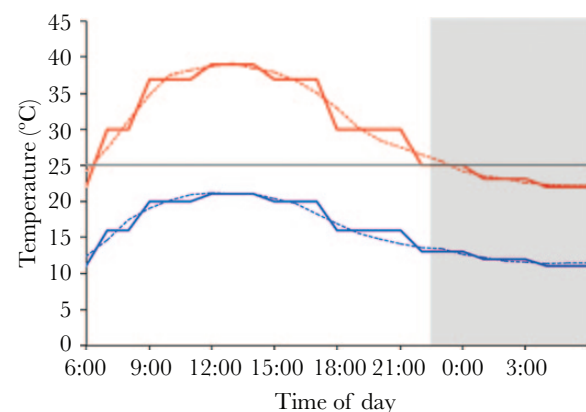
Figure 1

Hourly thermal regimes at which wasps were held between trials on host walking traces in Experiment 2 (solid lines; red—"hot," blue—"cool," gray—"control"). For the hot and cool treatments, dotted lines show averaged hourly field temperature data on which the experimental regimes were based (see Methods). White background—photophase; Shaded background—scotophase.

Experienced wasps were trained on a first arena of host walking traces at 25 °C, collected into a PCR tube, and immediately assigned to 1 of the 3 temperature regimes (hot, cool, or control). In parallel, an equal number of naïve wasps were assigned to each of the temperature treatments without training them on a contaminated arena. On the same day (1-5 h after training) and during the 3 subsequent days (day 1: 18–30 h, day 2: 42–54 h, day 3: 66–78 h after training), subsets of wasps from each temperature/experience treatment combination were removed from the temperature treatments, acclimated at 25 °C for 15 min, and tested on an arena of host walking traces at 25 °C between 8:45 and 15:30 h (each wasp was retested only once). The above time intervals (hereafter, "test intervals") thus represent the duration that wasps were held at the temperature regimes (for experienced wasps, the time since the end of training). After excluding wasps that did not show an arrestment response to host cues (4.1%), were lost (3.7%) or died before they were tested (2.2%), a total of 459 wasps were included in the analysis (241 control, 108 cool, 110 hot).

Statistical analysis

For Experiment 1, we tested the dependence of the arrestment response (residence time, linear speed, and tortuosity index) on experience status (naïve vs. experienced), temperature treatment, and experience × temperature treatment interaction. We also statistically controlled for the time of day at which the wasp was tested by including it as a factor in the model when it was significant. However, we do not focus on its effects in our interpretation of the results, because it was likely due to simple differences in parasitoid activity levels over the course of the day (Colazza and Pompanon 1994).

For Experiment 2, we tested the dependence of the arrestment response on experience, temperature regime, test interval, and time of day. Because our hypotheses include the possibility that the effect of experience over time could depend on temperature, we also tested for all possible 2- and 3-way interactions between experience, temperature, and test interval. When significant 3-way interaction effects were present, we subsequently analyzed each temperature treatment separately to aid in the interpretation of the effects of other factors.

For both experiments, parametric survival models assuming a Weibull distribution ("survreg"; Crawley 2007; Therneau 2014) were fitted to residence time data (which were not normally distributed, typical of time-to event data), and linear models (assuming a normal error distribution) were fitted to velocity and tortuosity index data. Model fit was assessed with residual plots. Significance of each factor in the survival analyses was determined using likelihood ratio tests (LRTs) comparing the full model with and without the factor in question, starting with higher-order interactions (Crawley 2007). Significance levels for factors in the linear models were derived directly from F-tests in the simplified model (containing only significant factors); statistical information given for nonsignificant factors is from when they are added to the simplified model.

All statistical analyses were carried out with R software, version 2.15.1 (R Core Team 2013).

RESULTS

Experiment 1: the effect of thermal stress on memory consolidation

Short-term temperature stress did not affect the wasps' behavioral expression of memory retention—none of the characteristics of the arrestment response were significantly influenced by an interaction between temperature treatment and experience (Table 1). As expected, experienced *T. basalis* females previously trained on host walking traces showed a significantly less pronounced arrestment response than naïve females, spending less time on the arena, walking faster, and having a lower tortuosity index (Figure 2; Table 1). Temperature treatment had a significant effect on linear velocity independent of experience, with wasps walking slower after having previously been at 40 °C than at 10 °C, although this effect was not present for residence time or tortuosity index (Figure 2; Table 1).

Experiment 2: the effect of thermal stress on memory retention

The residence time of *T. basalis* females was influenced by a significant 3-way interaction between experience, temperature, and test interval (LRT, df = 5, $\chi^2 = 11.17$, and $P = 0.048$). The predictions of the survival model containing the 3-way interaction (overall model significance: $\chi^2 = 180.75$, df = 11, and $P < 0.0001$) are plotted through the observed residence times of *T. basalis* females in Figure 3. Under the control temperature regime, the residence time of experienced females increased relative to that of naïve females with increasing time elapsed since their unrewarded experience (i.e., there was a significant experience × test interval interaction; Table 2), with the difference between the predicted residence time of naïve versus experienced wasps decreasing from 133.3% (301.00 ± 22.34 s vs. 129.04 ± 10.68 s; Kaplan–Meier estimates ± SE) at 1 h to 47.2% (290.29 ± 20.79 vs. 197.20 ± 15.35) after 72 h (Figure 3). Under the cool temperature regime, the residence time of naïve wasps decreased with increasing test interval, while it increased under the hot temperature regime (Table 2; Figure 3). However, these 2 temperature treatments differentially affected the residence time of experienced and naïve wasps (relative to the control temperature regime)—the residence time of experienced wasps increased less over time than would be expected if they showed the same response to the temperature treatments as the naïve wasps (Table 2; Figure 3). The responses to time interval of naïve and

Table 1

Statistical comparison of arrestment response characteristics of *T. basalis* females on host walking traces in Experiment 1, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), exposure temperature between patches, the interaction of the 2 factors, and the time of day at which the test took place

Measurement	Factor	Test statistic$_{df}$	P
Residence time[a]	Experience	$\chi_1^2 = 41.13$	<0.0001
	Temperature	$\chi_2^2 = 3.34$	0.19
	Experience × temperature	$\chi_2^2 = 1.84$	0.40
	Time of day	$\chi_1^2 = 9.16$	0.0025
Linear velocity[b]	Experience	$F_{1,235} = 66.17$	<0.0001
	Temperature	$F_{2,235} = 6.45$	0.0019
	Experience × temperature	$F_{2,233} = 0.90$	0.41
	Time of day	$F_{1,234} = 1.75$	0.19
Tortuosity index[b]	Experience	$F_{1,236} = 5.28$	<0.0001
	Temperature	$F_{2,234} = 1.26$	0.29
	Experience × temperature	$F_{4,232} = 1.23$	0.30
	Time of day	$F_{1,236} = 12.25$	<0.001

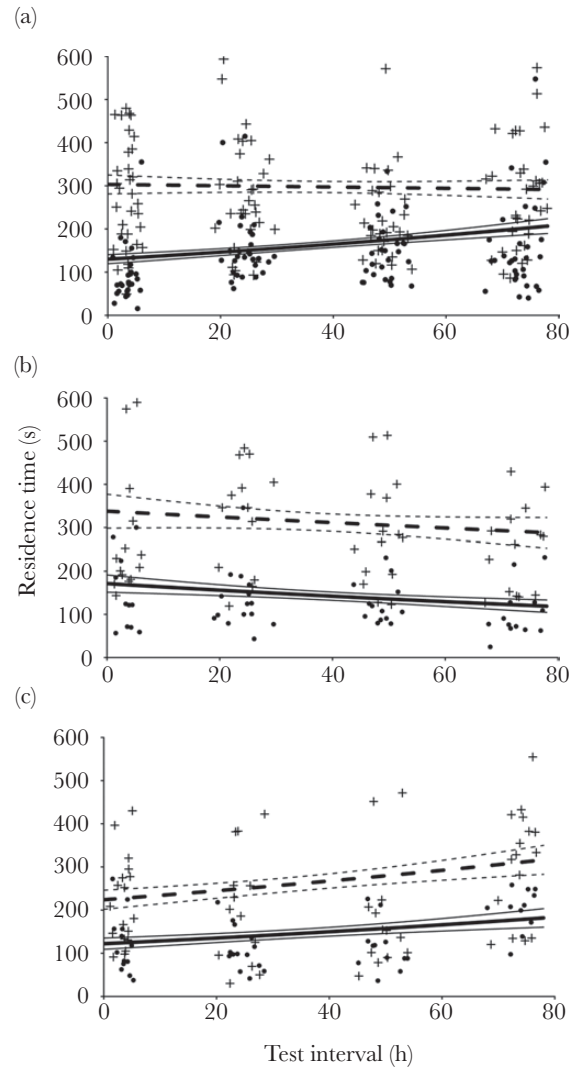[a]Significance was assessed with LRTs comparing survival models.
[b]Significance was assessed with F-tests comparing nested linear models
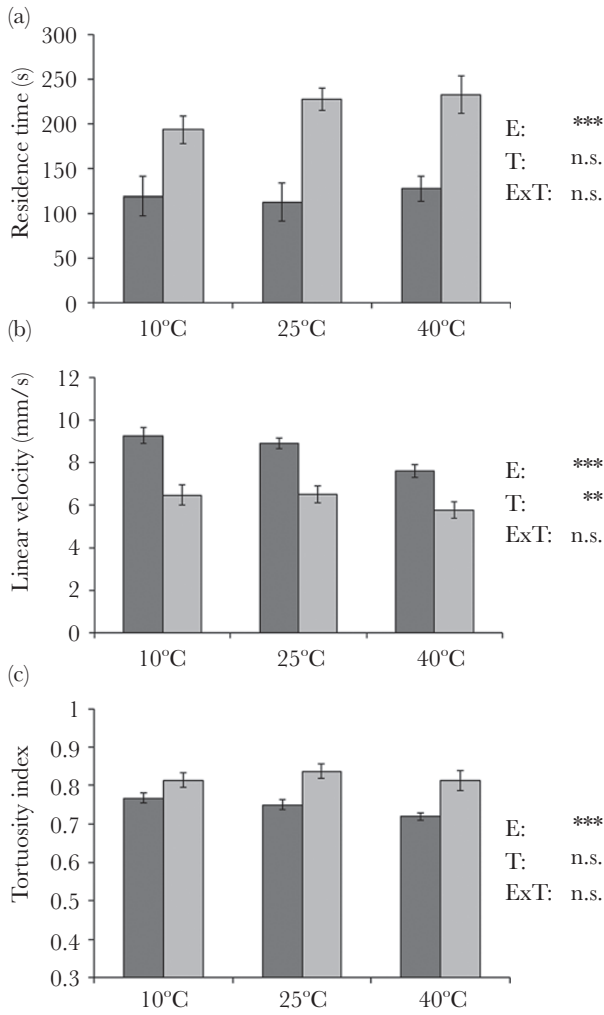
Figure 2

The mean (±SE) (a) residence time, (b) linear velocity, and (c) tortuosity index of experienced (dark bars; tested 1.25 h after being trained on another arena of host walking traces) and naïve (light bars; no previous experience) *T. basalis* females tested on host walking traces at 25 °C when held at 1 of 3 temperatures (10 °C, 25 °C, or 40 °C) during the hour between training and testing (experienced wasps) or for 1 h before testing (naïve wasps). In each panel, legends show the statistical significance of experience (E), temperature (T), and their interaction (E × T). n.s.—not significant ($P >$ 0.05), ** $P < 0.01$, ***$P < 0.001$. Refer to Table 1 for detailed statistical information.

Figure 3

The residence times of *T. basalis* females tested on host walking traces after being held at (a) constant temperature of 25 °C, (b) a low-temperature regime, or (c) a hot-temperature regime for 0–80 h (see Figure 1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid—experienced; dashed—naïve) show Kaplan–Meier estimates (±SE) of a survival model fitted to the data (see Results).

experienced wasps thus had similar slopes under the cool and hot regimes (i.e., nonsignificant time interval × experience interaction; Table 2), and meant that the difference between naïve and experienced wasps was just as pronounced 3 days after the unrewarded experience as the day on which they were trained.

Linear velocity was significantly higher for experienced *T. basalis* females than for naïve females, but did not change with increasing test interval and was not affected by temperature or any of the interactions between these factors (Table 3; Figure 4). Tortuosity index was significantly lower for experienced wasps than naïve wasps, and was influenced by a significant interaction between temperature regime and test interval (Table 3; Figure 5). Modelling each temperature treatment separately revealed a marginally nonsignificant increase in tortuosity index over time in the control ($F_{1,238} = 1.743$, $P = 0.083$) and hot ($F_{1,107} = 1.92$, $P = 0.058$) temperature regimes,

and a marginally nonsignificant decrease with increasing test interval in the cool temperature regime ($F_{1,105} = 1.86$, $P = 0.066$) (Figure 5).

DISCUSSION

Depending on the stability and possible adaptive plasticity of memory retention in the face of thermal stress, stressful temperatures could affect the ability of ectotherms to adjust behaviors such as time allocation as they move through heterogeneous environments. Contrary to our first hypothesis, our results suggest that *T. basalis'* memory is stable in the face of short-term exposure to stressful temperatures at the limits of what this parasitoid would encounter in nature. However, in line with our second hypothesis, the behavioral expression of memory persisted when wasps were held at high and low temperature regimes over the period of several days,

Table 2

Statistical comparison of the residence time of *T. basalis* females on host walking traces after being held at 3 different thermal regimes, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), the test interval spent in the temperature regime, the interaction of the 2 factors, and the time of day at which the test took place

Temperature	Factor	χ^2_{df}	P
Control	Experience		
	Test interval		
	Experience × test interval	$\chi^2_1 = 7.07$	0.0078
	Time of day	$\chi^2_1 = 0.19$	0.66
Cool	Experience	$\chi^2_1 = 59.50$	<0.0001
	Test interval	$\chi^2_1 = 4.19$	0.041
	Experience × test interval	$\chi^2_1 = 0.60$	0.44
	Time of day	$\chi^2_1 = 0.45$	0.50
Hot	Experience	$\chi^2_1 = 31.73$	<0.0001
	Test interval	$\chi^2_1 = 9.27$	0.0023
	Experience × test interval	$\chi^2_1 = 0.046$	0.83
	Time of day	$\chi^2_1 = 0.73$	0.39

Significance was assessed with LRTs comparing survival models. Significance of the main effects was not tested when the interaction between them was significant.
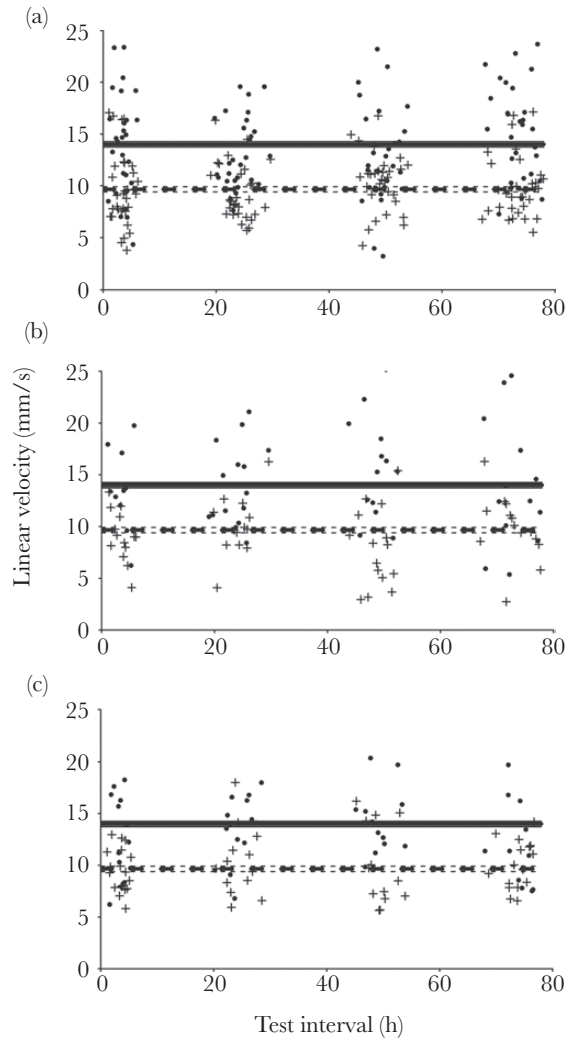
Table 3

Statistical comparison of the linear velocity and tortuosity index of *T. basalis* females on host walking traces, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), the temperature regime at which they were held before testing, the test interval spent in the temperature regime, the interactions between these factors, and the time of day at which the tests took place

Measurement	Factor	F_{df}	P
Linear velocity	Experience	$F_{1,457} = 141.30$	<0.0001
	Temperature	$F_{2,455} = 0.37$	0.69
	Test interval	$F_{1,456} = 0.48$	0.49
	Experience × temperature	$F_{4,453} = 1.25$	0.29
	Experience × test interval	$F_{2,455} = 0.24$	0.79
	Temperature × test interval	$F_{3,454} = 0.70$	0.70
	Experience × temperature × test interval	$F_{6,451} = 0.77$	0.59
	Time of day	$F_{1,456} = 0.87$	0.35
Tortuosity index	Experience	$F_{1,452} = 35.08$	<0.0001
	Temperature	$F_{2,452} = 6.06$	0.0025
	Test interval	$F_{1,452} = 2.86$	0.091
	Experience × temperature	$F_{2,450} = 1.24$	0.29
	Experience × test interval	$F_{1,451} = 0.60$	0.44
	Temperature × test interval	$F_{2,452} = 4.00$	0.019
	Experience × temperature × test interval	$F_{3,449} = 1.03$	0.38
	Time of day	$F_{1,451} = 0.67$	0.41

Significance was assessed with *F*-tests comparing nested linear models.

Figure 4

The linear velocity of *T. basalis* females tested on host walking traces after being held at (a) constant temperature of 25 °C, (b) a low-temperature regime, or (c) a hot-temperature regime for 0–80 h (see Figure 1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid—experienced; dashed—naïve) show predictions (±SE) of a linear model fitted to the data (see Table 3).

whereas the wasps appeared to forget their unrewarded experience when held at a moderate temperature. To our knowledge, this is the first experimental evidence that realistic temperature stress could modify time allocation strategies via an effect on memory retention.

It is well established that brief exposure to extreme low temperatures soon after learned experiences causes amnesia in several

ectotherm organisms, because memories are often the least stable in their early phases (reviewed in Margulies et al. 2005; Hoedjes et al. 2011). In addition to disrupting early ASM phases, cold shock can affect the consolidation of later, more stable forms of memory (van den Berg et al. 2011). Although it has not previously been tested in insects, it is plausible that short-term heat stress could also disrupt memory consolidation (Beck and Rankin 1995). If these effects extend to naturally occurring temperatures, a very hot afternoon or a cool night could induce full or partial amnesia in parasitoids and, as a result, a poor estimate of the reliability of host-associated cues. In our study, however, brief (1 h) low-temperature stress at the lower limit of what could actually be experienced during *T. basalis*'s foraging period in nature (10 °C) did not affect the intensity of the wasps' subsequent arrestment response when applied directly following an unrewarded experience (Figure 2). Similarly, brief high-temperature stress (40 °C) did not disrupt the behavioral expression

(a)

(b)

(c)

Test interval (h)
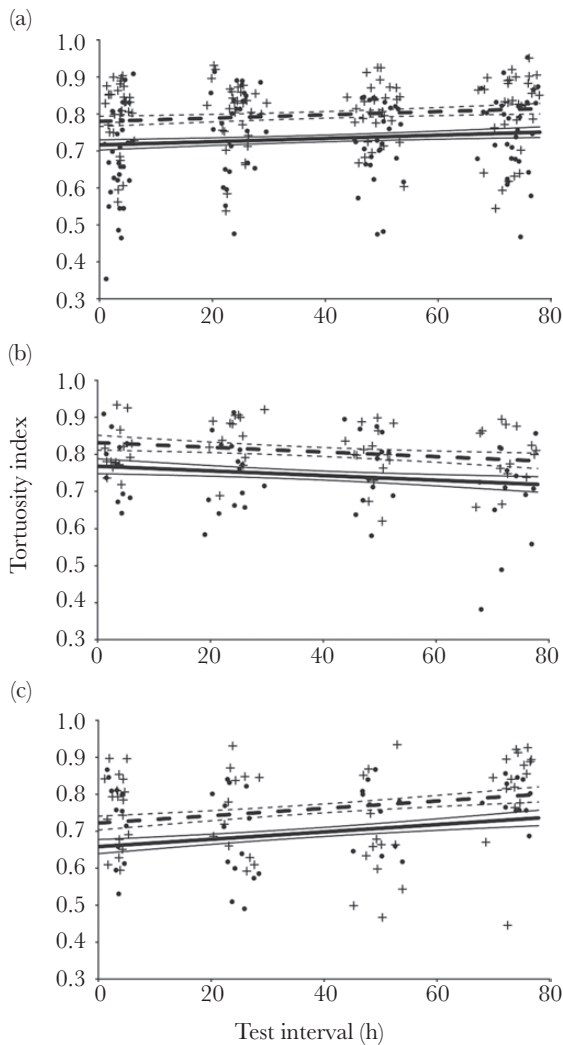
Figure 5
The tortuosity index of *T. basalis* females tested on host walking traces after being held at (a) constant temperature of 25 °C, (b) a low-temperature regime, or (c) a hot-temperature regime for 0–80 h (see Figure 1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid—experienced; dashed—naïve) show predictions (±SE) of a linear model fitted to the data (see Table 3).

of memory consolidation (Figure 2), as was also found by Teskey et al. (2012) in the snail *Lymnaea stagnalis* when testing the effect of temperature stress on its siphon withdrawal reflex. It seems unlikely then, that short-term thermal stress in nature could induce amnesia by acting as a constraint on memory consolidation.

Although consolidating memories when facing thermal stress would be important in the short term, it may be equally important for animals to retain, and then eventually forget unrewarded experiences in the longer term. *Nezara viridula*, the primary host species of *T. basalis*, is multivoltine, attacks a wide range of host plants in different environments over the course of a season, and uses different plants for mating and oviposition (Todd 1989; McPherson and McPherson 2000). Thus, the reliability of adult walking traces as an indicator of host egg presence may be variable among habitats. Among-habitat variability in the reliability of host-associated cues

would make it important for parasitoids to "reset" their sensitivity to walking traces (i.e., forget an unrewarded experience) when moving between low-quality and high-quality habitats, in order to avoid underinvesting foraging time in a new, potentially profitable habitat (Colazza et al. 2014). As expected, the residence time of *T. basalis* with a previous unrewarded experience on patches of host walking traces tended to increase over the 80 h testing period (relative to naïve controls) when held at a moderate temperature between trials, suggesting that "forgetting" took place (Figure 3a). When held at either hot or cool daily temperature cycles, however, there were differential effects of thermal stress on naïve and experienced individuals over time, when compared with the control temperature treatment. In both the cool and hot treatment, this resulted in a consistently lower residence time for experienced wasps compared with naïve wasps across the testing period, indicating that forgetting did not take place under these regimes. This result could indicate an adaptive modification of the parasitoids' memory window, or a physiological constraint imposed by temperature (sensu Moiroux et al. 2014)—2 possibilities we will now discuss.

There is some theoretical (Dunlap et al. 2009) and empirical (Pravosudov and Clayton 2001; Friedrich et al. 2004; Orsini et al. 2004) support for the idea that memory duration should be maximized when organisms are in poor state, because the fitness cost of not responding correctly (in this case, leaving the patch sooner) to a familiar stimulus (host walking traces) is higher when the organism is stressed (Dunlap et al. 2009). Hence, a parasitoid that is physiologically stressed during a heat wave or a series of cold nights, and whose ability to reproduce depends on finding hosts before energy reserves are depleted, is better off remembering the correct response to the previously unreliable cue, rather than forgetting and then having to relearn that the cue is unreliable. An alternative to this adaptive explanation is that temperature simply acts as a constraint on forgetting. If, as recent studies suggest, forgetting is indeed an active physiological process (Berry et al. 2012; Hadziselimovic et al. 2014) and the rates of underlying cellular and neurological mechanisms are temperature-dependent, one might expect forgetting to follow a traditional thermal performance curve (Angilletta et al. 2002). Thus, as observed in our study, the rate of forgetting would be maximal within the parasitoid's "comfortable" thermal range, and lowest at the extremes. The only other studies of memory retention in the face of thermal stress were conducted in the gastropod *L. stagnalis*, where there was also evidence of longer memory retention when exposed to stressful high or low temperatures after a learned experience (Sangha et al. 2003; Teskey et al. 2012). The authors provided primarily non-adaptive, constraint-based explanations for the observed effects (i.e., "priming" of neurons at high temperatures, disruption of molecular mechanisms associated with forgetting at low temperatures) and did not consider an adaptive explanation in either case.

Obviously, thermal stress would not affect the time allocation strategies of foraging animals only via its effect on memory retention. Residence time of foraging animals while responding to host-associated cues in nature would depend on current temperature (van Damme et al. 1990) (which, for simplicity, we held constant in this study). Previously experienced temperatures interacting with aspects of physiological state that are unrelated to memory can also affect parasitoid residence time (e.g., van Baaren et al. 2005; Bourdais et al. 2012). For example, we observed an increase in the residence time of naïve wasps spending increasing amounts of time in the "hot" temperature regime, and the reverse trend under the "cool" regime (Table 2). This could represent gradual physiological acclimation of the parasitoids to the stressful thermal regimes

over time, and supports the idea that heat waves or cool periods could affect the subsequent time allocation strategies of parasitoids in additional ways that do not directly depend on their previous foraging experience (see Hance et al. 2007).

Our results showed varying degrees of correspondence between our 3 measurements of the behavioral expression of memory (residence time, linear velocity, and tortuosity index), especially when comparing the short-term and long-term experiments. In the short-term experiment (Experiment 1), differences in residence time between treatments generally mirrored changes in linear velocity and tortuosity index. However, in the long-term experiment, the increase in the residence time of experienced wasps relative to naïve controls over time (i.e., "forgetting") (Figure 3a) was not accompanied by the expected decrease in linear velocity and increase in tortuosity index (Figures 4 and 5). The reasons for this discrepancy are unclear and deserve further investigation, although it indicates that the forgetting observed, and how it was modulated by temperature exposure, was not directly related to simple changes in locomotor activity.

Another factor preventing a detailed mechanistic interpretation of our results is an inability to relate the observed effects to the associated phases of memory consolidation, and the type of learning involved (see Margulies et al. 2005; Hoedjes et al. 2011). Because the timing and duration of different memory phases is species-specific in insects, it is unknown whether the thermal stress in our first experiment was applied during the ASM phase or rather during a more stable memory phase (e.g., anaesthesia-resistant memory or long-term memory; see Margulies et al. 2005). Furthermore, it is currently unclear whether the utilization of host-associated cues by *T. basalis* constitutes habituation (a decreased sensitivity to host-associated cues with increased exposure), associative learning (the association of walking traces with the absence of hosts; Takasu and Lewis 1996), or a combination of a habituative and associative learning processes. Experiments are currently being performed to explore these possibilities. In any case, we suspect that the findings of our study could extend to both habituative and associative learning, because both learning types appear to have common underlying genetic and neurological mechanisms (Duerr and Quinn 1982; Engel and Wu, 1996; Cho et al. 2004; Asztalos et al. 2007) and could thus have similar responses to thermal stress.

Predicting the responses of ecosystems to climate change necessitates a thorough understanding of how the foraging strategies of organisms will change when exposed to stressful temperatures. Any thermal effect that modulates time allocation strategies is likely to affect attack rates, which would directly affect predator-prey or parasitoid-host population dynamics (Murdoch 1994; Hassell 2000). Although more straightforward metabolic effects of temperature can certainly explain a certain proportion of the variation in time allocation and attack rates under different temperature regimes (e.g., Brown et al. 2004; Le Lann et al. 2011; Sentis et al. 2013; Le Lann et al. 2014), a consideration of realistic temperatures' effects on learning and memory is critical, because they may change foraging strategies in ways that cannot be predicted by changes in metabolic rates. Future studies in this area should attempt to 1) distinguish between the hypotheses for temperature-dependent memory windows that we have put forward in this paper, 2) relate differences in memory retention at different temperatures to realized fitness gains, and 3) examine whether ectotherm organisms can use behavioral thermoregulation to buffer any possible thermal constraints on memory retention.

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