Temperature limits trail following behaviour through pheromone decay in ants

Louise van Oudenhove & Elise Billoir & Raphaël Boulay & Carlos Bernstein & Xim Cerdá

Abstract In Mediterranean habitats, temperature affects both ant foraging behaviour and community structure. Many studies have shown that dominant species often forage at lower temperature than subordinates. Yet, the factors that constrain dominant species foraging activity in hot environments are still elusive. We used the dominant ant Tapinoma nigerrimum as a model species to test the hypothesis that high temperatures hinder trail following behaviour by accelerating pheromone degradation. First, field observations showed that high temperatures (> 30°C) reduce the foraging activity of T. nigerrimum independently of the daily and seasonal rhythms of this species. Second, we isolated the effect of high temperatures on pheromone trail efficacy from its effect on worker physiology. A marked substrate was heated during 10 min (five temperature treatments from 25°C to 60°C), cooled down to 25°C, and offered in a test choice to workers. At hot temperature treatments (>40°C), workers did not discriminate the previously marked substrate. High temperatures appeared therefore to accelerate pheromone degradation. Third, we assessed the pheromone decay dynamics by a mechanistic model fitted with Bayesian inference. The model predicted ant choice through the evolution of pheromone concentration on trails as a function of both temperature and time since pheromone deposition. Overall, our results highlighted that the effect of high temperatures on recruitment intensity was partly due to pheromone evaporation. In the Mediterranean ant communities, this might affect dominant species relying on chemical recruitment, more than subordinate ant species, less dependent on chemical communication and less sensitive to high temperatures.

Keywords Ant foraging behaviour · Bayesian inference · Mechanistic model · Mediterranean ant community · Tapinoma nigerrimum · Trail pheromone

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Introduction

In eusocial insects, workers' foraging behaviour is expected to maximise the overall colony energy intake (Oster and Wilson 1978). The total biomass of food retrieved by a colony directly results from the intensity and duration of foraging activity (Cole et al. 2008). This foraging effort fluctuates both in the number of foragers involved and in the time they spend foraging (Cole et al. 2008). Temporal and spatial patterns of foraging activity are affected by both intrinsic and extrinsic factors (Hölldobler and Wilson 1990). On the one hand, endogenous characteristics of the colony such as maturity (presence of brood: Portha et al. 2004; age of workers: Traniello 1989) or genetic background (Cole et al. 2010) might influence the foraging

schedule. On the other hand, exogenous factors that include both biotic interactions such as predation (Whitford and Bryant 1979), competition (Gordon 1992; Brown and Gordon 2000) or food availability (Bernstein 1975; Brown and Gordon 2000) and abiotic conditions such as microclimate (Marsh 1985; Bucy and Breed 2006; Azcárate et al. 2007; Chong and Lee 2009) are expected to influence the foraging activity of many ant species.

In Mediterranean ecosystems, among the climatic variables that influence ant foraging, temperature is probably the most important (Azcárate et al. 2007). External temperatures are especially decisive for foraging activity since extreme temperatures affect the muscular control and survival of individuals (Cerdá et al. 1998; Lighton and Turner 2004; Maysov and Kipyatkov 2009). In several ant communities, behaviourally dominant species that displace subordinate species at mild temperatures are less tolerant to stressful temperatures (Cerdá et al. 1998; Bestelmeyer 2000). This dominance-thermal tolerance trade-off contributes to community structure promoting species coexistence (Retana and Cerdá 2000; Lessard et al. 2009; Wittman et al. 2010). Temperature variations might thus reduce competitive exclusion through physiological differences between dominant and submissive species. In addition, competitive superiority is often linked to the use of chemical communication in territorial and foraging behaviour (Hölldobler and Carlin 1987; Fellers 1987; Savolainen and Vepsäläinen 1988), and dominant species advantage might be limited by the efficiency of their chemical communication.

Many ant species use chemical communication to exploit food sources efficiently (Hölldobler and Wilson 1990). When a scout discovers a resource, it may lay a chemical trail while returning to the nest in order to guide nestmates to the resource. The chemical trail can be reinforced by each returning forager. Trail pheromones are generally composed of a mixture of relatively volatile compounds (see Morgan 2009 for review). For instance, the trail pheromone of the ant Tetramorium caespitum L. is a mixture of two pyrazines (2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine, Attygalle and Morgan 1983). The rate at which the trail pheromone fades out may depend on temperature (as it occurs for sex pheromones, McDonough et al. 1989; Ono 1993). Ruano et al. (2000) performed phylogenetic analyses establishing that ant species foraging at hot temperatures do not rely on chemical recruitment. However, to our knowledge, trail pheromone sensitivity to high temperature, and its consequence on foraging activity has not been yet documented.

In this study, we analysed how foraging activity and trail-following behaviour vary according to temperature. We hypothesized that the high temperature sensitivity of trail pheromones would constrain ant foraging activity. To test this hypothesis, we used a widely distributed Mediterranean ant species Tapinoma nigerrimum as a model system. Previous studies have shown that this species uses mass recruitment with chemical trails (Cerdá et al. 1989). First, we analysed how foraging activity varies through the year and during the day as a function of ground temperature in the field. Second, we devised a laboratory experiment to discriminate the effect of high temperature on trail pheromone from the thermal stress on individuals. We tested thereby whether pheromone volatility limits ant recruitment in a range of temperatures found in nature. Third, we built a mechanistic mathematical model in order to explore the relationship between ant foraging activity and the rate of pheromone decay with temperature. In contrast with standard statistical models, our model was designed for predictive purposes and to allow a broader extrapolation on what occurs in nature out of the range of the studied values. Our approach, which is based on Bayesian inference of parameter estimates, is a flexible and powerful method especially when models involve multiple events, or dynamical processes (Ellison 2004), as may be the case with workers recruitment during ant foraging.

Materials and methods

Model species and study sites

T. nigerrimum is a Mediterranean ant species, widespread in coastal areas (Bernard 1983; Cerdá et al. 1989). Colonies are polygynous and polydomous, composed of extensive nests with many entrances interconnected by aboveground worker trails (Cerdá et al. 1989). Colonies are relatively populous (tens of thousands of workers) with polymorphic workers (López et al. 1997) ranging from 2.5 to 5.1 mm length (Gómez and Espadaler 1998). It is an opportunistic species that mainly collects liquid resources such as aphid-honeydew or nectar, but also feeds upon dead insects (Cerdá et al. 1989; Gómez and Espadaler 1998). T. nigerrimum mainly adopts a collective foraging strategy by mass recruitment with chemical trails between the nest and food sources, but does not use exploratory chemical paths (Couret and Passera 1979). The chemicals contained in the gaster of T. nigerrimum workers are identical to those of its twin species Tapinoma simrothi (Hefetz and Lloyd 1983; L. van Oudenhove and A. Lenoir, unpublished results) suggesting that T. nigerrimum trail pheromone is mainly composed of iridodials and iridomyrmecins (Simon and Hefetz 1991).

Colony fractions of 1,000–5,000 workers were collected in the south of Spain (Doñana National Park, Huelva province; Sierra Nevada National Park, Granada province). They were reared in the lab in plastic boxes, the bottoms of

which were coated with plaster to maintain humidity. Small tubes with water and cotton wool also contributed to nest humidity. Room temperature remained constant at 25°C±1°C. Ants were fed three times a week with meal worms (Tenebrio molitor).

The effect of ground temperature on foraging activity in the field

Data from a field study conducted previously in Canet de Mar (Barcelona, NE Spain) were analysed by focusing on the influence of temperature on the foraging behaviour of T. nigerrimum. The study site was a savannah-like grassland of Hyparrhenia hirta with Foeniculum vulgare, Brachypodium retusum and some very scattered pines (Pinus pinea); it was at 50 m above sea level and 750 m away from the coastline; the climate was of a Mediterranean type (Cerdá et al. 1989, 1998).

A colony of T. nigerrimum was selected, and its foraging activity was estimated from the number of foraging ants on a trail connecting the nest to a food source (aphids in a pine tree). The number of workers moving in both directions in a trail and crossing a mark (a line with a thread 2 cm high located at 50 cm from the nest entrance) was recorded for 3 min of each hour for 24 h on 11 days between April and November 1985 (Cerdá et al. 1989). Each day, the sampling was done on the trail which was the most active at the beginning of the sampling period (at 7 h). Together with the hourly measurement of activity at trail, ground surface temperature was measured at the nest entrance with glass-headed thermocouples and a Univolt DT-830 multimeter.

Data were analysed by fitting generalized linear models (GLM) using the R software (R Development Core Team 2010). The response variable was the number of ants on the trail. To take into account the auto-correlation due to the circadian rhythm, we modelled the daily cycle introducing as auxiliary independent variables $H_{cos} = \cos \left(2p \frac{Hour}{24} \right)$ and
$H_{sin} = \sin \left(2p \frac{Hour}{24} \right)$ as proposed in Crawley (2002). The other explanatory variables were the day of field observation (11-level factor), the temperature (continuous) and their interaction. To deal with the overdispersion of data, we used a GLM with Quasi-Poisson error distribution. To give some flexibility to the effect of temperature on the response variable, we first introduced temperature effect as a second-degree polynomial. Non-significant effects were then progressively removed comparing the resulting scaled deviances with an F test. Level reductions of the day variable were then tested by grouping chronologically successive days. Non-significant levels were removed according to an F test on the scaled deviances. Day variable with remaining levels was defined as the day-group variable.

The effect of temperature on trail following behaviour

The effect of temperature on pheromone persistence and ant ability to follow a trail was analysed in the lab. First (phase 1), a 2-day fasting colony was connected to an unlimited food source (1:3 v/v honey/water) through two narrow (25×2×0.3 cm) glass bridges (Fig. 1a). During the next 30 min after the first ant started to explore the bridge, ants crossing a marked line (mark a in Fig. 1a) on the second bridge (X) were counted during 1 min every 5 min. We call initial efficacy (eff_0) the mean number of outbound ants that crossed the bridge per minute for the first 30 min. In a second step (phase 2), the X glass bridge and a control, unmarked bridge (U) were maintained during 10 min at a given temperature (25°C, 30°C, 35°C, 40°C, 50°C or 60°C) in an universal precision oven (J.P. Selecta, precision error <2%). Both bridges were then cooled down during 5 min to room temperature (25°C). During this phase, the tested colonies remained connected to the first bridge without food. In the third step (phase 3), ants were offered access to a Y-shaped device constituted with the bridges X and U as the diverging branches, both leading to identical empty containers; both were at room temperature (Fig. 1b). The location of both bridges (left or right side of the Y) was randomly chosen at each trial. To prevent the use of visual cues by the ants, the experimental setup was surrounded by dark panels and was illuminated with homogeneous light. The number of ants crossing either bridge during 1 min was counted every 2 min during 30 min after the connection of the bridges. Experiments were performed with four colony fragments, and two replicates per nest were conducted for each
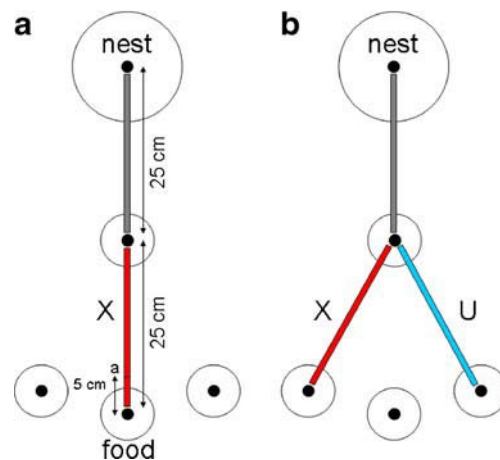

Fig. 1 Diagram of the experimental design to study the trail following behaviour in Tapinoma nigerrimum. a Phase 1: pheromone deposition by workers crossing bridge X (counted at point a during 30 min); b phase 3: Y-shaped device to test ant choice. X bridge was marked during phase 1 while U bridge is unmarked. Both bridges were warmed during the temperature treatment (phase 2) and cooled down to room temperature (25°C) before the test

treatment temperature. The experiments were performed in a randomised order for each nest.

We fitted two GLMs. In both cases, the response variable was the number of ants that crossed bridge X (success) and U (failure) during 1 min. The first GLM focus on the very first choice of workers to avoid confounding effects appearing afterwards, such as interactions with unladen returning workers. Since ants tend to explore the empty container before travelling back, we estimated that 10 min was a good approximation of the first workers choice. The first GLM thus tests whether or not temperature had an effect on the percentage of ants choosing bridge X in the first 10 min. Treatment temperature was considered as a categorical predictor variable to avoid assumptions on the shape of its effect. The significance of each level was tested by changing the contrast matrix. The second GLM aimed to identify the factors involved in the probability of choosing bridge X. The explanatory variables were the nest (four-level factor), the initial efficacy (continuous), the temperature of treatment included as a continuous variable (continuity was justified by the first GLM), and the time elapsed since the connection of the bridges (continuous). We used a GLM with a logit link function and a quasi-binomial error distribution. We built a full model that contained all variables and interactions between them. We first tested level reductions of the "nest" variable. Then, we progressively removed the non-significant effects until obtaining the most parsimonious model. Model selection was based on the comparison of the resulting scaled deviances with an F test.

The mechanistic model

Our mechanistic model aimed at specifying the nature of the relationship between temperature and the probability of choosing a branch in a dynamic process. We estimated model parameters using Bayesian inference with the data of the previous experiment.

Firstly, for each experimental replicate j ($j \in [1, 48]$), we assumed the initial efficacy eff_j to be an estimate of the initial pheromone concentration (phase 1). Then, we suggested that the concentration of pheromone on a branch $C_{t, j}$ is a function of the initial concentration eff_j, and the time elapsed at the different temperatures. As in previous studies on pheromone evaporation, we assumed an exponential decay of the trail pheromone concentration (Beckers et al. 1993; Jeanson et al. 2003).

$$C_{t;j} = eff_j \, e^{-at} \qquad (1)$$

where α is the inverse of the mean lifetime of the trail pheromone. It will be considered as the evaporation velocity of the chemicals. According to the kinetic theory

of gases commonly used to model biological processes (Gillooly et al. 2001; Brown et al. 2004), the average molecular kinetic energy is proportional to the absolute temperature (in degrees K) ($\frac{1}{2}mv^2 = \frac{3}{2}k_BT$, where k_B is Boltzmann's constant, m and v are respectively the mass and the velocity of the particle and T is the absolute temperature). The velocity of each particle is hence proportional to the square root of the absolute temperature. We assumed that the evaporation velocity of the pheromone depends likewise on the temperature: $a = b\sqrt{T} + T_{ref}$, with ß and T_{ref} being constants specific to the pheromone composition. Note that particular units used to express temperatures do not matter provided that T_{ref} and T are expressed on the same scale. We used Celsius degrees. So, we explicited Eq. 1:

$$C_{t;j} = eff_j \, e^{-bt\sqrt{T} + T_{ref}} \qquad (2)$$

In our experiment, pheromone decayed differently during the last two phases. During phase 2, the bridges were exposed during 10 min (t_h) at the experimental temperature (T). The remaining concentration after this phase is thus $C_{0;j} = eff_j \, e^{-bt_h\sqrt{T} + T_{ref}}$:

During the test (phase 3), bridges were exposed i min at room temperature ($T_e = 25°C$). Pheromone concentration is thus $C_{i;j} = C_{0;j}e^{-bi\sqrt{T_e} + T_{ref}}$:

The amount of pheromone on a branch determines the probability of choosing a given branch in a two branches choice. Deneubourg et al. (1990) suggested the following function to quantify the probability of choosing branch X, prob(X), given two branches X and U.

$$prob(X) = \frac{(k + N_X)^n}{(k + N_X)^n + (k + N_U)^n} \qquad (3)$$

N_X (resp. N_U) represents the number of ants that previously passed on branch X (resp. U). Parameter n determines the degree of non-linearity of the choice, e.g. a high value of n means that even slight differences between branches lead to high probability of choice. Parameter k corresponds to the degree of attraction attributed to an unmarked branch.

The number of ants that passed on a branch is considered equivalent to the pheromone concentration on this branch. In our system, no ant passed on bridge U ($N_U = 0$) and the pheromone concentration on bridge X, at time i, is $C_{i, j}$. Equation 3 becomes:

$$prob(X)_{i;j} = \frac{(k + C_{i;j})^n}{(k + C_{i;j})^n + k^n} \qquad (4)$$

We assumed that the effective number of ants choosing bridge X ($X_{i, j}$) was a realisation of a binomial function with success probability $prob(X)_{i, j}$ and $Tot_{i, j}$ trials, where $Tot_{i, j}$

is the total of ants coming out of the nest at time i for replicate j.

To estimate the parameters of the mechanistic model, we used Bayesian inference. All parameters were considered as probability distributions (Online resource ESM_1, Fig. S1). Bayesian inference consists in updating from chosen prior probability distributions to posterior probability distributions given the data (Spiegelhalter et al. 1996; Ntzoufras 2009). Practically, Bayesian inference was performed using Markov Chains Monte Carlo (MCMC) algorithms with the rjags R package (Plummer 2009). Some details on the inference are provided in Online resource (ESM_1) and implementation code is available upon request to the authors.

When attempting to estimate simultaneously the four parameters involved in the proposed mechanistic model, according to the Gelman and Rubin diagnostics (Gelman and Rubin 1992; Brooks and Gelman 1997), the Bayesian algorithm failed to reach convergence. This was due to strong correlation between parameters, which suggested overparameterization of the model. To reduce the number of parameters to estimate, we tested the values proposed by Deneubourg et al. (1990) on the basis of an empirical fitting: $k \approx 20$ and $n \approx 2$. Hence, we fitted three simplified models, by fixing respectively k, n or both. In these three cases, the convergence of the estimation process was successful. We then relied on the Deviance Information Criterion (DIC, Plummer 2009) to identify the best model.

Results

The effect of ground temperature on foraging activity in the field

Temperature had a significant nonlinear effect on the foraging activity of $T.$ $nigerrimum$ depending on both the hour of the day and the day of the year. The best statistical model included the effect of the hour, the day-group (see Online resource ESM_2 for the establishment of the day-group variable), and temperature as a quadratic function (Table 1). Daily variations explained part of the variability in foraging activity fluctuations. The endogenous circadian oscillations represented by the two variables H_{cos} and H_{sin} also had a significant influence. Even taking into account the circadian rhythm, the relation between foraging activity and ground temperature followed a parabola. For low temperatures ($<25°C$), raising temperature increased foraging activity while the opposite was registered for high temperatures ($>30°C$). However, significant interactions between the day and both temperature and squared temperature revealed that the exact shape of the parabola varied between days with maximum activity ranging

Table 1 The influence of soil temperature, circadian rhythm and day of observation on the foraging activity of $Tapinoma$ $nigerrimum$ (number of ants on the trail)

Effect[1]	Df	Resid. Df	F	P value
H_{sin}	1	240	31	<0.001
H_{cos}	1	239	222	<0.001
Day-group	6	233	97	<0.001
Temperature	1	232	154	<0.001
Temperature^2	1	231	174	<0.001
Day-group : Temperature	6	225	9	<0.001
Day-group : Temperature^2	6	219	4	0.001

Analysis of deviance table of the selected GLM (family, quasipoisson; link, log; response, number of ants). Terms were added sequentially (first to last). H_{sin} and H_{cos} refer to the daily variations (see Materials and methods). Day-group represents the seasonal variations (group of days in different period of the year)

between 18°C and 30°C. Among the 11 days, some showed similar patterns and could be regrouped into seven day-groups (Fig. 2). For five day-groups (day-group A: 20/04, day-group C: 01/06 and 01/07, day-group D: 13/07, 24/07 and 07/08, day-group E: 21/08 and day-group G: 04/11), temperature was a good predictor of foraging activity. However, for day-groups B (19/05) and F (16/09 and 14/10), temperature effect was not significant.

Temperature effect on trail following behaviour

Treatment temperature had a significant effect on ants first choice ($F_{5,234} = 35$, $p < 0.001$). During the first 10 min, ants chose preferentially bridge marked (X) after temperature treatments lower than 40°C but failed to discriminate between bridges after higher treatment temperatures (Fig. 3). However, the only significant difference between two adjacent temperatures was between 25°C and 30°C ($t = 3.213$, $p = 0.002$). The effect of temperature on choice probability was rather progressive (Fig. 3), which made more appropriate the consideration of temperature effect as a continuous variable.

The analyses of ant choice variation across time specified the effect of different variables on the persistence of trail pheromone. The most parsimonious GLM (see Online resource ESM_2) included the effect of the logarithm of the temperature of treatment, the time elapsed, the initial efficacy (eff_0), the group of nests (nests have been reduced without loss of precision to a two-level factor, see Online resource for details), the interaction between temperature and time, and the interactions of eff_0 with temperature, time and nest (Table 2). The higher the treatment temperature, the weaker was the preference for bridge X. Time passed since the connection
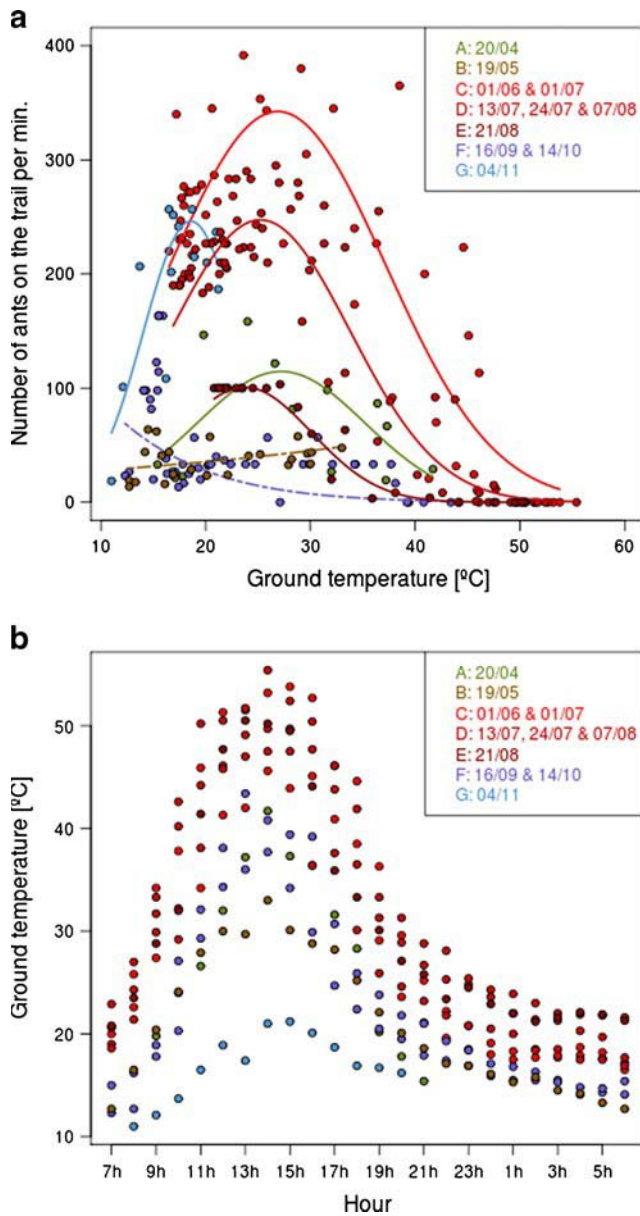
Fig. 2 a Number of *Tapinoma nigerrimum* foragers on a trail in field conditions according to soil temperature. Field observations (points) are hourly recorded in a day (24 points per day, 13 points for 20/04 and 04/11). Days showing similar activity patterns were grouped. Colours differentiate day-groups from April to November (see insert). Activity-temperature curves (lines) are predicted from the GLM. Dot-dashed lines stand for non-significant temperature effects. b Temperature variations according to the hour of record throughout the sampling days. Equivalent days regarding ants foraging activity are represented with similar colours (see insert)
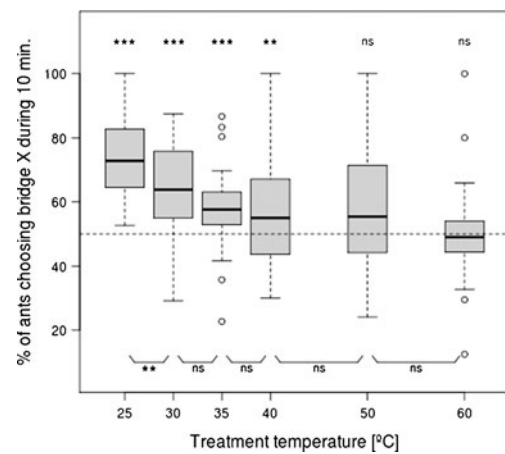

Fig. 3 Box plot of the proportion of *Tapinoma nigerrimum* workers choosing the previously marked bridge (X) as a function of the temperature treatment. This graph represents the ants' first choice since it groups data recorded less than 10 min after the start of the experiment (connection of bridges X and C) for all nests (n=40 per temperature treatment). The top and bottom of the box mark the limits of ±25% of the data; the median value is displayed as a line. Vertical lines mark the acceptable range (±1.5*IQD, where IQD is the interquartile distance). Temperature effect is estimated from the GLM by changing the contrast matrix. The significance of choice probability for each temperature is indicated above the boxes. Differences between two treatments in the probability of choosing are represented at the bottom of the plot. (***$p<0.001$; **$p<0.01$; *$p<0.05$, ns=$p>0.05$)

of the Y-shaped device also reduced significantly the probability that ants choose the previously marked bridge (Table 2). The positive interaction between temperature and time effect prevented the underestimation of the choosing probability for high temperature and time values. For high values of eff_0, the decrease in ants' choosiness

due to increases in temperature and time elapsed was higher. The response varied between nests, but the coupled effect of temperature and time was independent of nest (Table 2).

Table 2 Choice of a pheromone marked or an unmarked bridge in a Y-shaped device, as a function of treatment temperature, initial efficacy (eff_0), nest group and time elapsed since the connection of the bridges

	Estimate	Std. error	t value	p value
nest (group 1)	3.735	0.507	7.374	<0.001
nest (group 2)	4.576	0.522	8.769	<0.001
eff_0 (group 1)	0.027	0.008	3.328	<0.001
eff_0 (group 2)	0.011	0.009	1.294	0.196
time	−0.080	0.021	−3.895	<0.001
log(temperature)	−1.038	0.142	−7.335	<0.001
eff_0 : time	−0.0002	0.00008	−2.348	0.019
eff_0 : log(temperature)	−0.004	0.002	−1.999	0.046
time : log(temperature)	0.022	0.006	3.830	<0.001

Estimation of each variable in the linear predictor of the GLM with quasibinomial family. Dispersion parameter for quasibinomial family was 1.85 (estimated by dividing the Pearson residuals by the residual degrees of freedom)
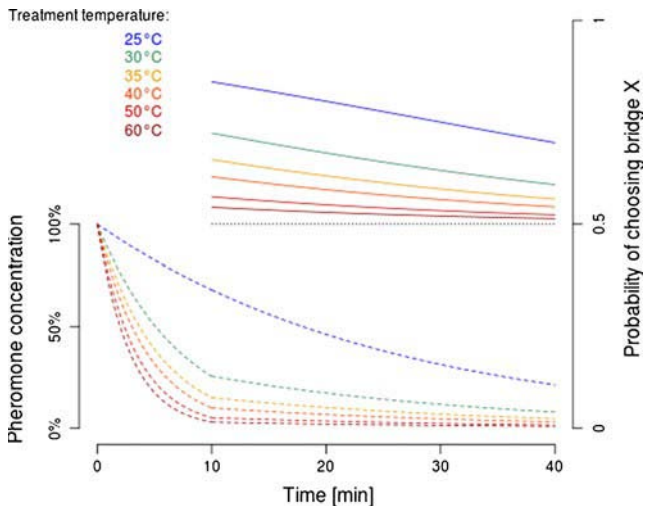
Fig. 4 Pheromone decay and its influence on ants' choice as predicted by the mechanistic model. Lower panel estimation of the pheromone concentration on the marked branch of a Y-shaped device. Upper panel probability of choosing the marked bridge

The mechanistic model

The minimum DIC was obtained for the simplified model fixing k at 20 and the parameters were estimated as (median [95% CI]): $T_{ref} = -24.56$ [−24.74; −24.34], $ß = 0.059$ [0.053; 0.065] and $n = 1.17$ [1.01; 1.33]. To check the goodness-of-fit of the mechanistic model, we compared observed and corresponding predicted data (Online resource Fig. S2). All observed values of X were reasonably predicted by the model with no clear outlier. The three simplified models led to not significantly different $ß$ estimates (0.056 [0.051; 0.062] when fixing n and 0.064 [0.059; 0.070] fixing both k and n), and to close T_{ref} estimates (−24.46 [−24.66; −24.20] fixing n and −23.74 [−23.98; −23.46] fixing k and n).

The model allows predicting the dynamics of pheromone concentration, a process difficult to access experimentally. The predictions concern both the time course of pheromone concentration depending on temperature (Fig. 4, lower part), and its direct influence on ants path choice (Fig. 4, upper part). The accordance between the predictions of our model and the experimental data (Online resource Fig. S2) suggests that the key processes involved were accounted for.

Discussion

The present study shows the importance of temperature on the recruitment behaviour in ants. Field observations highlighted the effect of temperature on the shape of the foraging activity curve. Behavioural assays demonstrated that the rate of pheromone decay depended on temperature. The nature of the dependence was successfully assessed by a mechanistic model.

The field results demonstrated that, as expected, T. nigerrimum's foraging activity depended strongly on ground temperature both at the daily and seasonal scales. In natural conditions, the shape of recruitment intensity according to temperature was unimodal. The peak of maximal activity estimated at 24°C in a previous study (Cerdá et al. 1998) varied throughout the year between 18°C in November and 30°C in June. This seasonal difference in temperature preference might be controlled by the interaction with other abiotic factors such as atmospheric pressure, humidity or light radiation (Marsh 1985; Azcárate et al. 2007; Chong and Lee 2009), or by the variation of the biotic environment such as resource availability, competition or predation pressure (Brown and Gordon 2000; Wittman et al. 2010). The intrinsic state of the colony, dependent on the reproductive cycle of the species, might also be an essential factor influencing both the needs and the foraging ability (number and age of workers) of the colony (Hölldobler and Wilson 1990). However, in spite of the variation in seasonal preferences, ground temperature remained a good predictor of surface activity in T. nigerrimum.

The behavioural assays on branch choice clearly showed that high temperatures accelerated pheromone decay, probably through the evaporation or degradation of its components. High temperature affected trail pheromone orientation function. Other abiotic factors such as the foraging substrate also influence recruitment efficiency (Jeanson et al. 2003). Unfavorable environmental conditions might thus be essential in collective foraging processes since they affect chemical communication between foragers. This modification of the information transfer interplays with an amplification of individual preferences. Indeed, regarding high temperatures, the direct effect on ant physiology is essential (Hölldobler and Wilson 1990). In Mediterranean environment, hot temperatures might eventually represent a mortality risk (Cerdá et al. 1998). The effect of temperature on foraging patterns might thus result from the interplay between individual physiology and pheromone decay. In mass recruiting species, pheromone stability might thus have co-evolved with ants' thermal tolerance. Indeed, in species commonly feeding on stable resources, such as T. nigerrimum that exploits aphid honeydew, pheromone persistence is essential to foraging efficiency. In this species, if the pheromone is sensitive to high temperature, the costly synthesis of heat shock proteins necessary for thermotolerance (Gehring and Wehner 1995) would not be adaptive.

Our mechanistic model allowed us to assess pheromone functionality from ants' behaviour. The simple mechanism we suggested (Eq. 2) described the contribution of high temperatures on pheromone decay. We linked pheromone evaporation to ants' choice with the reference model of

Deneubourg et al. (1990). Our model could be parameterised by Bayesian inference on experimental data, making predictions reliable. Moreover, our MCMC method raised an interesting point about the path choice model of Deneubourg et al. (1990). This model provides an accurate description of ants' choice given the intensity of the chemical signal. Practically, ants' preferences depend on pheromone concentration and two parameters that control the choice non-linearity. When running iterations to estimate parameters by Bayesian inference, these two parameters appeared positively correlated: the concentration threshold for trail efficacy is higher when the choice is more sensitive to the amount of pheromone. Besides, our estimates were rather different from the values obtained when fitting the exploratory behaviour of the Argentine Ant Linepithema humile (Deneubourg et al. 1990) ($n \approx 2$, $k \approx 20$) or the recruitment trail of Lasius niger Beckers et al. (1993) ($n \approx 2$, $k \approx 6$). We estimated a lower degree of choice nonlinearity (fixing $k = 20$, $n \approx 1.17$; fixing $n = 2$, $k \approx 39.59$). Theoretically, low nonlinearity suggests a poor ability to select the best food source (Nicolis et al. 2003). Nevertheless, since T. nigerrimum displayed good trail following proficiency, the difference might rather rely on a divergence of experimental procedures. Indeed, they differed on many aspects from the measure of initial pheromone concentration to the starvation state of the colonies (Hangartner 1969).

At the community level, differences between ant species' thermal tolerance is often invoked to explain species coexistence (Cerdá et al. 1998; Bestelmeyer 2000; Lessard et al. 2009; Wittman et al. 2010). T. nigerrimum is a dominant species (Cerdá et al. 1997; Blight et al. 2010). In Mediterranean ant communities, behaviourally dominant species are less tolerant to high temperatures, and thermal stress might even disrupt the expected dominance hierarchy (Cerdá et al. 1997). As behavioural dominance is closely linked to numerical dominance (Savolainen and Vepsäläinen 1988; Davidson 1998), competitive superiority might be mediated by the recruitment process. A loss in recruitment efficiency might thus affect the competitive capacity thereby contributing to the disruption of dominance hierarchy at high temperatures. Moreover, in some species the same compounds cause both recruitment and alarm behaviour. For instance, in T. simrothi, closely related to T. nigerrimum, the same exudate is used for both trail-following and defensive behaviour according to its concentration although the concentration threshold to induce alarm behaviour is much higher (about ten times) than for trail-following (Hefetz and Lloyd 1983; Simon and Hefetz 1991). Very high pheromone concentrations might thus be necessary to evoke in ants a high level of aggression. If high temperatures affect pheromone concentration as we show here, it might be very difficult to trigger ants' aggressiveness when soil is hot. On the other hand, subordinate species use other foraging strategies less dependent on chemical communication (e.g. group recruitment or individual foraging) and are less sensitive to high temperatures (Ruano et al. 2000). Therefore, their foraging efficiency remains unaffected by high temperature. So, in Mediterranean communities where temperature shows important seasonal and daily variations, the pheromone thermal decay affects preferentially dominant species, and can be considered as one of the mechanism ensuring the persistence of the inferior competitors.

To conclude, our study provides experimental evidence of the effect of high temperatures reducing trail pheromone concentration. The behavioural response of foraging ants regarding daily and seasonal variations in temperature might thus be assigned to restrictions on both ants' physiology and their communication system. By directly affecting individuals, high temperatures reduce forager density and therefore reduce pheromone accumulation on trails. Reduced accumulation of trail pheromones combines with faster evaporative rates at higher temperatures to further reduce foraging activity. The value of our mechanistic model is to enable explicit quantitative predictions based on first principles. It is particularly adapted to the experimental setting we developed where ants were not exposed to high temperatures but trail pheromone was.

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