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The smell of change: warming affects species interactions mediated by chemical information

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Abstract

Knowledge of how temperature influences an organism's physiology and behaviour is of paramount importance for understanding and predicting the impacts of climate change on species' interactions. While the behaviour of many organisms is driven by chemical information on which they rely on to detect resources, conspecifics, natural enemies and competitors, the effects of temperature on infochemical-mediated interactions remain largely unexplored. Here, we experimentally show that temperature strongly influences the emission of infochemicals by ladybeetle larvae, which, in turn, modifies the oviposition behaviour of conspecific females. Temperature also directly affects female perception of infochemicals and their oviposition behaviour. Our results suggest that temperature-mediated effects on chemical communication can influence flows across system boundaries (e.g. immigration and emigration) and thus alter the dynamics and stability of ecological networks. We therefore argue that investigating the effects of temperature on chemical communication is a crucial step towards a better understanding of the functioning of ecological communities facing rapid environmental changes.

Keywords: chemical communication, climate change, insects, metabolic theory of ecology, nontrophic interactions, temperature

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Introduction

Climate change affects the diversity, composition, structure and functioning of ecological communities (Pereira *et al*., 2010). Understanding underlying mechanisms is a major challenge for ecologists because multiple species' interactions can either strengthen or weaken the direct effects of climate change on individual species (Tylianakis *et al*., 2008; Sentis *et al*., 2013a). Recent efforts have been devoted to the development of a general mechanistic framework to understand and predict the consequences of climate change on trophic interactions and food-web dynamics (Fussmann *et al*., 2014; Gilbert *et al*., 2014; Sentis *et al*., 2014). However, this framework does not currently account for the effects of temperature on nontrophic interactions (i.e. nonfeeding interactions) such as habitat modification, predator interference and facilitation. These nontrophic interactions are extremely common in ecosystems and contribute significantly to the dynamics and composition of ecological networks (Kéfi *et al*., 2012).

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Most animals and plants rely on chemical information (i.e. infochemicals) to detect resources, conspecifics, competitors and natural enemies (Cardé & Millar, 2004; Peñuelas & Staudt, 2010). Infochemicals thereby play a crucial role in both trophic and nontrophic interactions (Mondor *et al*., 2004; Johansson & Jones, 2007; Yuan *et al*., 2009). However, the effects of climate change on the emission and perception of infochemicals as well as their consequences for species' interactions, populations and community dynamics remain poorly understood (Holopainen *et al*., 2013). Indeed, very little information exists on the effects of temperature on ecological interactions mediated by infochemicals (but see Mcneil, 1991), and there is no general framework to integrate these effects into ecological networks.

As chemical compounds are metabolic products (Blomquist & Bagnères, 2010), we expect the infochemical emission rate to scale with metabolic rate under some assumptions described below. The effects of temperature and body mass on metabolic rate have been extensively quantified and, more recently, integrated into a general metabolic framework known as the metabolic theory of ecology (MTE) (Brown *et al*., 2004). According to MTE, metabolic rate scales with temperature as follows:

$$I = i_0 M^{b_i} e^{-E_i/kT} \quad (1)$$

where I is the standard metabolic rate, i_0 is a normalization constant independent of body size (M) and temperature (T) (Brown *et al.*, 2004), b_i is an allometric exponent (0.75), E_i is the activation energy for metabolism (0.65 eV; Brown *et al.*, 2004) and k is Boltzmann's constant (8.62×10^{-5} eVK^{-1}). The value of the activation energy gives the slope of the relationship between temperature and metabolic rate. According to the Universal Temperature Dependence hypothesis (Gillooly *et al.*, 2001), this value (i.e. 0.65 eV) should be similar for most species and biological rates. We thereby expect that the emission of infochemicals would follow the same temperature dependence as metabolic rate, that is the mean activation energy (E) should equal 0.65 eV. However, the assumption that infochemical emission rate scale with metabolic rate may not hold in some cases where, for instance, infochemicals are temporally stored in glands and later actively released (e.g. sex, trail and alarm pheromones). We thus expect Eqn 1 to be reliable predictor in cases where infochemical are passively released by the producer (i.e. not stored). We therefore propose the MTE model as a null model for infochemical emission rate. Differences between predictions from Eqn 1 and empirical observations can therefore be used to examine the temperature dependence of additional mechanisms such as infochemical sequestration and active release. Because many biological rates, including interaction rates (e.g. feeding rate), generally conform to MTE (Burnside *et al.*, 2014), recent studies have used this theoretical framework to predict the consequences of climate change on species' physiology, behaviours, interactions and populations (Sentis *et al.*, 2013b, 2014; Gilbert *et al.*, 2014). Estimating the activation energy value for the temperature dependence of infochemical transmission is therefore a crucial step towards the integration of chemical-mediated interactions into temperature-dependent network models.

Most organisms have evolved sophisticated olfactory systems to detect subtle variations in infochemical information (Cardé & Millar, 2004; Hansson, 2014). Indeed, slight changes in the quality or quantity of infochemicals can alter mate choice (Johansson & Jones, 2007), prey defensive behaviour (Mondor *et al.*, 2004) and resource location by herbivores and natural enemies (Yuan *et al.*, 2009). We therefore hypothesize that, when temperature affects the quantity and/or composition of infochemicals synthesized and emitted by an organism (the emitter), other organism(s) (i.e. the receiver) would detect these changes and adjust their behaviour accordingly (Fig. 1a,b). Temperature may also directly affect the perception and response of an organism to infochemicals through its influence on the cognitive abilities of the receiver (Fig. 1c; Van Damme *et al.*, 1990). Altogether, these patterns suggest that the effects of temperature on both the producer and the receiver should be investigated simultaneously to determine the outcome on chemical communication.

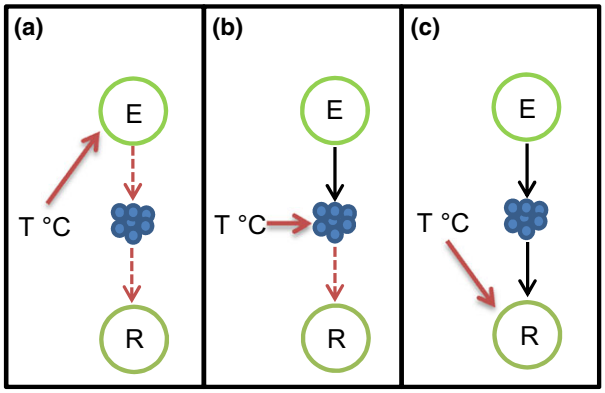

Fig. 1 Schematic representation of possible temperature effects on ecological interactions mediated by chemical information. The cluster of small blue circles represents infochemicals; the large green circles represent the individuals involved in the interaction, where E is the emitter that emits the infochemicals and R is the receiver that perceives the infochemicals (in some cases, E can also be R). Black arrows indicate the direction of information flows from the emitter to the receiver. Solid and dashed red arrows indicate direct and indirect temperature effects, respectively. (a) Temperature directly affects the emitter and triggers indirect effects on infochemicals and the receiver. (b) Temperature directly affects infochemicals and triggers an indirect effect on the receiver. (c) Temperature directly affects the receiver. Temperature may also directly affect two or more of these three components simultaneously (not shown for the sake of clarity).

The aim of this study was to determine the effects of temperature on the transmission (i.e. emission and perception) of infochemicals and the behavioural responses to them, using ladybeetles as a model system. As is the case with many other invertebrates, ladybeetles rely on infochemicals to assess the presence of conspecific and heterospecific individuals (Magro *et al.*, 2007; Blomquist & Bagnères, 2010). Infochemicals are important for the assessment of resource patch quality and strongly influence the oviposition behaviour of ladybeetles (see below for details). Under laboratory conditions, we (1) investigated the effects of temperature on the composition and quantity of infochemicals emitted by ladybeetle larvae (Fig. 1a; direct effect), (2) measured the consequences of infochemical changes on the oviposition response of conspecific females (Fig. 1a; indirect effect) and (3) measured the direct effect of temperature on the female oviposition response to conspecific larval infochemicals (Fig. 1c). We did not investigate the direct effect of temperature on the chemistry

of larval infochemicals once emitted (Fig. 1b) because infochemicals in our experimental system are mainly long-chained hydrocarbons that are stable molecules delivering a long-lasting signal (Hemptinne *et al.*, 2001). These are unlikely to be affected by the small experimental temperature range and the short duration of the experiments.

We found that temperature greatly modulates the emission and perception of infochemicals as well as the oviposition response to infochemicals. We discuss how these effects can influence important population processes such as immigration and emigration, and thus affect the dynamics and stability of ecological communities. Given the pervasiveness of infochemicals and their critical role in trophic and nontrophic interactions, we expect temperature effects on chemical communication to significantly influence ecological processes in most ecosystems.

Materials and methods

Biological system

Ladybeetles use infochemicals to assess the quality of oviposition sites (Magro *et al.*, 2007; Seagraves, 2009), as eggs and young larvae are highly vulnerable to starvation, cannibalism and predation (Hodek & Honěk, 1996). Larvae and adults of most ladybeetle species smear hydrocarbons on the substrate (Magro *et al.*, 2007), yet the significance of this behaviour, and thus the status of these infochemicals [i.e. pheromone (infochemical uses for intraspecific communication), kairomone (infochemical that benefits the receiver but not the emitter), allomone (infochemical that benefits the producer and harms the receiver), synomone (infochemical that benefits both the receiver and the emitter)], remains open for debate (Martini *et al.*, 2009). However, these long-chain hydrocarbon molecules (Hemptinne *et al.*, 2001; Magro *et al.*, 2007) provide a long-lasting signal (Hemptinne *et al.*, 2001) and females typically avoid laying eggs on substrates marked by conspecific and heterospecific competitors or predators (Doumbia *et al.*, 1998; Oliver *et al.*, 2006; Magro *et al.*, 2007; Seagraves, 2009). Ladybeetles therefore provide an excellent model to study the effects of temperature on chemical communication. The biological system under study consisted of the two-spot ladybeetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), a common predator of aphids in Europe, central Asia and North America (Hodek & Honěk, 1996), and one of its main prey, the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae).

Plant and insect production

All insects and plants were maintained at 20 ± 1 °C and under a 16L:8D photoperiod in an air-conditioned chamber (Liebherr® FKS 1802). *Acyrthosiphon pisum* was maintained on *Vicia faba* L. *Adalia bipunctata* came from a stock culture maintained in the laboratory and refreshed every year with wild individuals from the Toulouse region of France. Adults were reared in 5-L plastic boxes and fed three times a week with an excess of pea aphids. A piece of corrugated filter paper was added to each box to provide a suitable substrate for oviposition. Eggs were collected every day and incubated in Petri dishes in the same conditions outlined above.

Experiment 1. Temperature effects on larval infochemical emission

Production and extraction of larval infochemicals. Neonate coccinellid first-instar larvae were taken from the stock culture and isolated in 90 mm Petri dishes at 20 ± 1 °C and under a 16L:8D photoperiod. They were fed three times a week with an excess of pea aphids until they reached the fourth instar (L4). Freshly emerged L4 larvae (~24 h; mean weight ± SE: 10.32 ± 0.21 mg) were placed in individual Petri dishes containing a moistened filter paper and starved for 24 h to avoid contamination by frass when testing for infochemicals. Each larva was next carefully introduced into a glass tube (12 mm diameter; 75 mm long) topped by a cotton plug; these were kept in air-conditioned chambers (Liebherr® FKS 1802) at 15, 20, or 25 ± 1 °C. The temperatures chosen are within the range of nonstressful conditions commonly experience by *A. bipunctata* in their natural environment (Jalali *et al.*, 2010). After 24 h, larvae were removed and the tubes stored at −18 °C. For each temperature, four replicates of 30 tubes were produced. Four replicates of 30 tubes without larvae were handled similarly and used as blank controls.

To extract larval infochemicals, the 30 tubes from each replicate were pooled and washed with 1 ml of hexane (hexane Merck, HPLC grade for liquid chromatography). They were then washed a second time with 1 ml of hexane for maximum extraction. The 2 ml of extract from each replicate of tubes was pooled and partially evaporated to about 1 ml under a gentle stream of nitrogen. The remaining volume was transferred to a 1.5-ml autosampler vial (3855X Fisher Scientific) until complete evaporation (Millar & Sims, 1998). The dry residue was then dissolved in 50 µl of hexane. The four replicates of 30 control tubes were similarly processed. Samples were kept at 4 °C until analysis.

Chemical analyses. Chemical compound structures from the n-hexane extracts were determined using gas chromatography – mass spectrometry (GC-MS) (TSQ Quantum GC; Thermo Scientific, Waltham, MA, USA). The mass spectra were scanned from 60 to 450 m/z. Online data acquisition was carried out with XCALIBUR software (Thermo Finnigan, MA, USA). The names of identified compounds were abbreviated according to IUPAC (International Union of Pure and Applied Chemistry) nomenclature (see Appendix S1 in Supporting Information for methodological details).

Comparing the chemical profiles of larval infochemicals. We tested for both qualitative and quantitative differences between larval infochemical profiles among temperatures by evaluating the similarity between all pairs of possible

combinations using the Jaccard and the Bray–Curtis distances, respectively (Brosse *et al.*, 2011). To search for pairwise similarities among temperature regimes, nonmetric multidimensional scaling (NMDS) was performed using the 'metaMDS' function of the 'VEGAN' package (Oksanen *et al.*, 2008) of R statistical software (R Development Core Team, 2011). In NMDS, a stress parameter measures the degree to which between-temperature distances from the original data are preserved in a lower-dimensional ordination model (Clarke, 1993). We chose to use two dimensions to minimize the stress parameter (i.e. maximize the rank correlation between the calculated similarity distances and the plotted distances). To assess the effects of temperature on the qualitative and quantitative variations in larval infochemical profiles, two permutational MANOVAS were then performed (Mcardle & Anderson, 2001). This analysis uses distance matrices and was accomplished using the 'adonis' function of 'VEGAN'. Significance was verified with F-tests based on sequential sums of squares from 1000 permutations of the raw data. We next investigated the relationship between temperature and infochemical emission rate (μg of infochemical compounds. individual^{-1} day^{-1}) by fitting the MTE equation (eqn 1) to the data using a log-linear regression. As prior studies reported that the relationship between biological rates and temperature is sometimes hump shaped, we also fitted the following quadratic model to the data (Englund *et al.*, 2011):

$$I = i_0 M^{b_i} e^{l(-1/kT)+q(-1/kT)^2} \quad (2)$$

where I is the infochemical emission rate and i_0, l and q are fitted parameters. When l and q are significant and positive and negative, respectively, the quadratic model describes a concave downward (umbrella shaped) relationship with a thermal optimum (Englund *et al.*, 2011). We next determined which of the two models (linear or quadratic) best fit the data according to their AICc (Akaike information criterion corrected for small sample size) values.

Experiment 2. Consequences of infochemical changes mediated by temperature on oviposition behaviour

Female standardization. Neonate *A. bipunctata* larvae were reared in 175-cm^3 plastic boxes at 20 ± 1 °C and fed pea aphids *ad libitum* until pupation. Newly emerged adults (<24 h) were isolated and their sex determined. Couples (one male and one female) were then formed and isolated in 90-mm Petri dishes containing a piece of corrugated paper for oviposition. The feeding regime and climatic conditions were the same as for the larvae. Each day, eggs were removed and couples were transferred to clean Petri dishes and provided an excess of pea aphids. Ladybirds randomly selected for the experiments were 10–20 days old and had laid at least one egg replicate within the last 5 days. To standardize oviposition drive and hunger level, females were deprived of males and food for 16 h overnight before the beginning of the experiment.

Production at different temperatures of treatment filter papers marked with larval infochemicals. *A. bipunctata* neonate larvae were reared individually at 20 ± 1 °C in 50-mm Petri dishes and fed pea aphids *ad libitum* until they reached the fourth instar. Five larvae were then introduced into a 90-mm-diameter Petri dish lined with a 90-mm-diameter filter paper (FT-3-208-090; Sartorius Stedim Biotech S.A., Aubagne, France) and provided with pea aphids in excess. Petri dishes were immediately transferred to air-conditioned chambers (Liebherr® FKS 1802, Toulouse, France) set at 15, 20 or 25 ± 1 °C. After 24 h, *A. bipunctata* larvae and aphids were removed, and filter papers were brushed to remove frass and aphid remains. These treatment filter papers were kept at −20 °C for a maximum of 1 month. They were removed from the freezer 2 h before use so that their temperature would equalize with the experimental conditions. Experimental Petri dishes were then lined with these filter papers according to the corresponding treatments (see below for details).

Ovipositional response to larval infochemicals. This experiment was conducted at 20 °C. Mated females were submitted to four infochemical treatments: clean filter paper (control) and filter papers with larval infochemicals emitted at 15, 20 or 25 °C. At the beginning of the experiment, each female was randomly placed in a 90-mm Petri dish lined with the treatment filter paper and containing a piece of corrugated filter paper as oviposition substrate, a section of broad bean stem, pollen and pea aphids in excess. The proportion of females that reproduced and the number of eggs laid per female were recorded every hour for the first 12 h of the experiment, and then once again 24 h after the beginning of the experiment. There were 33 replicates of each treatment. Cox's proportional hazard model was used to test the effect of the four treatments on the proportion of ovipositing females, that is the probability of oviposition per unit of time. Cox's proportional hazard models were then performed to compare treatments two by two (e.g. control vs. larval infochemicals at 15 °C). Finally, an ANOVA was used to test differences among the four treatments in the number of eggs laid per female (i.e. clutch size) at the end of the 24-h period, and a *post hoc* Tukey test was performed to identify differences between means. Females that did not reproduce were not included in the ANOVA.

Experiment 3. Direct effects of temperature on ovipositional responses to infochemicals

We conducted a full-factorial experiment with females foraging at three temperatures (15, 20 and 25 ± 1 °C) and exposed to two infochemical treatments: clean filter paper as a control and filter paper with larval infochemicals emitted at 20 °C. We chose to use only infochemicals emitted at 20 °C to avoid confounding effects of temperature on infochemical emission. The method of female standardization, larval infochemical production, experimental set-up and data recording were similar to those used in Experiment 2. There were 30 ± 2 replicates per treatment. Cox's proportional hazard model was used to test the effects of infochemicals, temperature and their interaction on the proportion of ovipositing females over time. Because the interaction was statistically significant, Cox's proportional hazard models were then used to test the effect of

infochemicals for each temperature. Finally, a two-way ANOVA was used to test the effects of infochemicals, temperature and their interaction on the number of eggs laid per female (i.e. clutch size) at the end of the 24-h period. All data were analysed using R software (version 2.13.1; R Development Core Team, 2011). Females that did not reproduce were not included in the ANOVA.

Results

Experiment 1. Temperature effects on larval infochemical emission

A total of 61 compounds (17 linear alkanes, 31 monomethylalkanes, 10 dimethylalkanes and 3 unidentified compounds) were detected in the hexane extracts (Supporting Information; Table S1 and Fig. S1). The quantitative analysis of the larval infochemicals revealed an average (± SE) of 11.58 (± 0.42), 13.19 (± 0.54) and 22.83 (± 2.19) μg of extract in infochemicals collected in 24 h at 15, 20 and 25 °C, respectively (see Table S1). As predicted by MTE, these quantities increased exponentially with temperature (Fig. 2), and our estimate (± CI 95%) of the activation energy (0.49 ± 0.17 eV) is close to the value expected by MTE (0.65 eV; Brown *et al.*, 2004). However, a quadratic model best described the relationship between the quantities of infochemical produced in 24 h and temperature (AICc linear model = −1.69; AICc quadratic model = −3.18; Fig. 2).

Although the patterns of dissimilarity among larval infochemical profiles were more quantitative than qualitative, the NMDS clearly distinguished between temperature regimes in both cases (see Fig. S2). For the qualitative and quantitative compositions, a two-dimensional solution provided final stress values of 0.059 and 0.004, respectively, indicating a good preservation of the multidimensional between-temperature similarities in the reduced ordination space. Temperature had a significant effect on the qualitative and quantitative composition of larval infochemicals (permutational MANOVA; $F_{2,11} = 7.57$, $P = 0.001$, $R^2 = 0.63$ and $F_{2,11} = 22.04$, $P = 0.001$, $R^2 = 0.83$, respectively) and explained 63 and 83% of the differences in qualitative and quantitative composition between infochemical profiles, respectively. However, the diversity of compounds identified in larval infochemicals at 15, 20 and 25 °C was similar, with 58, 58 and 60 compounds, respectively (see Table S1). Most compounds (56 of 61) were found at all temperatures, and NMDS indicated that compounds 1, 7, 10, 40 and 57 contributed most to the qualitative partitioning of the different clusters associated with the three experimental temperatures (see Fig. S2a). Compounds 1, 2, 3, 4, 10, 40 and 57 contributed most to the quantitative partitioning of the different clusters (see Fig. S2b).

Experiment 2. Consequences of infochemical changes mediated by temperature on oviposition behaviour

The probability of oviposition per hour varied with infochemical treatments ($\chi^2 = 22.821$, df = 3, $P < 0.0001$, $n = 132$; Fig. 3). Two-by-two comparisons revealed that this probability was significantly higher in the control than in the treatments with infochemicals emitted at 15 °C ($\chi^2 = 6.13$, df = 1, $P = 0.0133$, $n = 65$), 20 °C ($\chi^2 = 7.29$, df = 1, $P = 0.0069$, $n = 62$) or 25 °C ($\chi^2 = 22.29$, df = 1, $P < 0.0001$, $n = 65$). Moreover, the

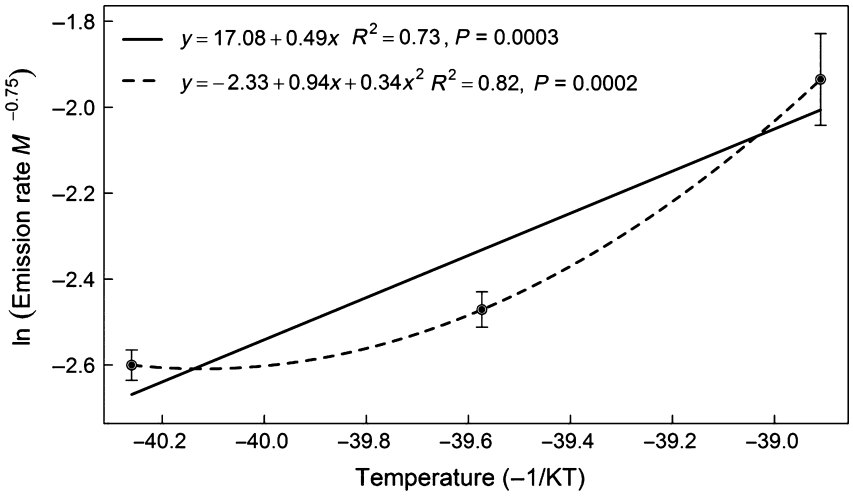

Fig. 2 Linear (solid line) and quadratic (dashed line) relationships between infochemical emission rate (mean ± SE) of *Adalia bipunctata* larvae and the inverse temperature (*T*, Kelvin) scaled with the Boltzmann constant (*k*). *M* represents the mean mass of *A. bipunctata* larvae (8.98 mg).

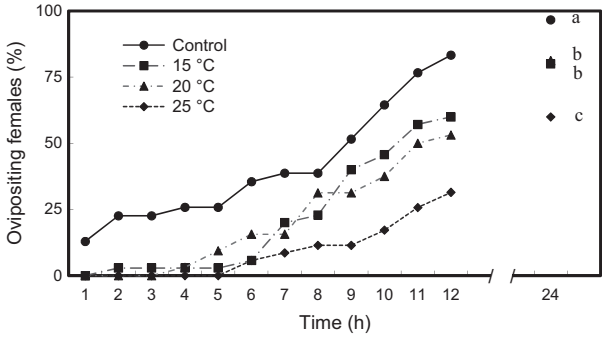

Fig. 3 Percentage of *Adalia bipunctata* female that laid eggs over a 24-h period in the control without larval infochemicals and in the treatments with infochemicals emitted at 15, 20 and 25 °C. Different letters on the right of the curves denote significant differences between the treatments ($P < 0.05$). Significance levels are from Cox's proportional hazard models.

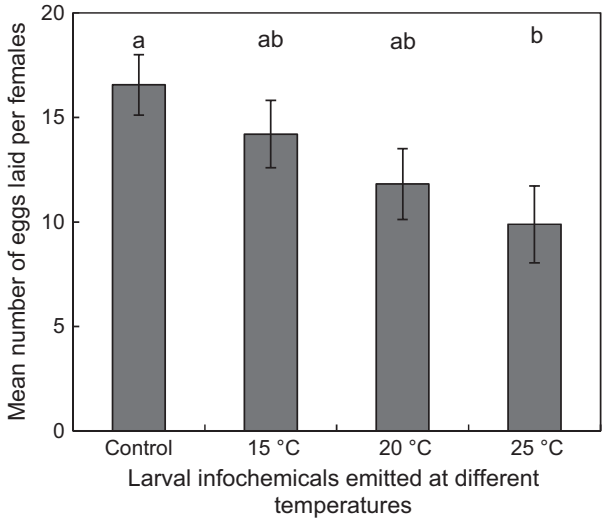

Fig. 4 Mean number (± SE) of eggs laid per *Adalia bipunctata* female after 24 h in the control without larval infochemicals and in the treatments with infochemicals emitted at 15, 20 and 25 °C. Bars with different letters are significantly different ($P < 0.05$). Significance levels are from *post hoc* Tukey tests.

probability of oviposition was lower in the treatment with infochemicals emitted at 25 °C than with infochemicals emitted at 20 °C ($\chi^2 = 4.76$, df = 1, $P = 0.0290$, $n = 67$) or 15 °C ($\chi^2 = 5.47$, df = 1, $P = 0.0193$, $n = 70$). There was no significant difference between infochemicals emitted at 20 °C and 15 °C ($\chi^2 = 0.02$, df = 1, $P = 0.8993$, $n = 67$).

The mean number of eggs laid by females after 24 h was significantly lower in the presence of conspecific larval infochemicals emitted at 25 °C compared to the control ($F_{3,128} = 2.96$, $P = 0.0349$, $n = 132$; Fig. 4).

Experiment 3. Direct effects of temperature on oviposition responses to infochemicals

The probability of oviposition per hour increased with temperature ($\chi^2 = 45.61$, df = 2, $P < 0.0001$, $n = 178$; Fig. 5) but was lower in the presence of larval infochemicals compared with the control ($\chi^2 = 10.06$, df = 1, $P = 0.0015$, $n = 178$; Fig. 5). However, the effect of infochemicals depended on temperature (interaction temperature regimes × infochemical treatments: $\chi^2 = 6.94$, df = 2, $P = 0.031$, $n = 178$): it was significant at 15 °C ($\chi^2 = 9.76$, df = 1, $P = 0.0018$, $n = 56$; Fig. 5a) and 20 °C ($\chi^2 = 7.29$, df = 1, $P = 0.0069$, $n = 62$; Fig. 5b), but not at 25 °C ($\chi^2 = 0.06$, df = 1, $P = 0.8062$, $n = 60$; Fig. 5c).

The mean number of eggs laid by *A. bipunctata* after 24 h increased with temperature ($F_{2,172} = 23.55$, $P < 0.0001$, $n = 178$; Fig. 6). It was lower in the infochemical treatments compared with the control ($F_{1,172} = 10.86$,

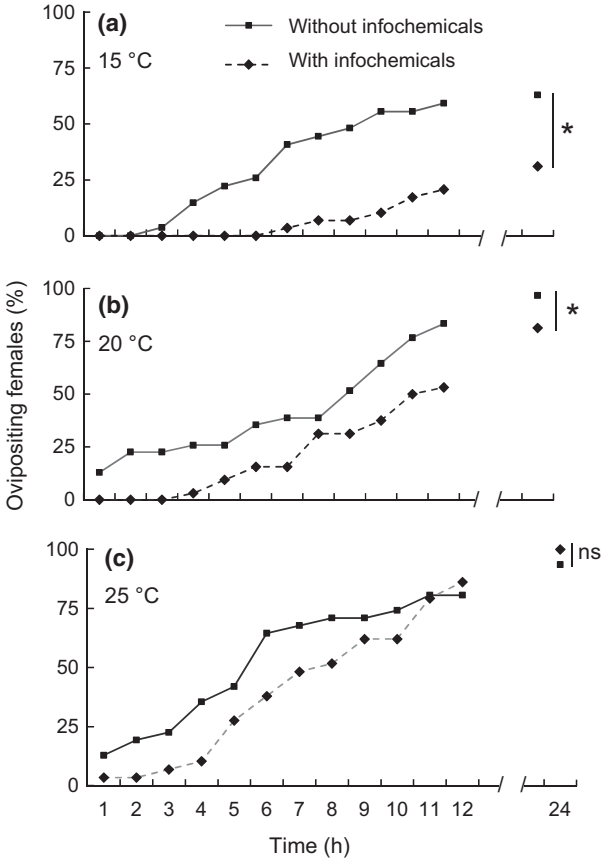

Fig. 5 Percentage of *Adalia bipunctata* females that laid eggs over a 24-h period with and without infochemicals (dashed and solid lines, respectively) at (a) 15 °C, (b) 20 °C and (c) 25 °C. Within temperature regimes, 'ns' stands for not significant and '*' indicates significant differences ($P < 0.05$) between the control without infochemicals and the treatment with infochemicals. Significance levels are from Cox's proportional hazard models.

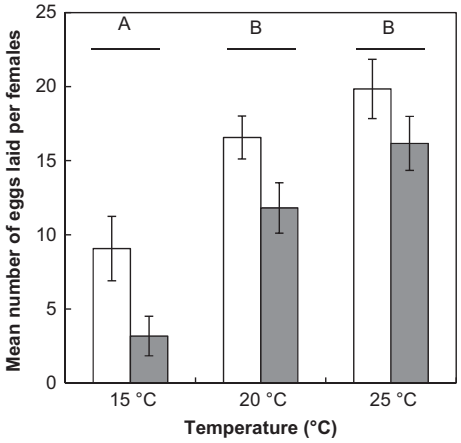

Fig. 6 Mean numbers (± SE) of eggs laid per *Adalia bipunctata* female after 24 h without or with infochemicals (open and grey bars, respectively) at (a) 15 °C, (b) 20 °C and (c) 25 °C. Different letters above bars denote significant differences between the temperature regimes ($P < 0.05$). Significance levels are from *post hoc* Tukey tests.

$P = 0.0012$, $n = 178$; Fig. 6), and the effect was similar among temperatures (interaction temperature regimes × infochemical treatments: $F_{2,172} = 0.20$, $P = 0.8228$, $n = 178$).

Discussion

Understanding how temperature influences physiology and behaviour of organisms is of paramount importance for predicting the impacts of climate change on species' interactions. While the behaviour of many organisms is driven by chemical information, the effects of temperature on infochemical-mediated interactions remain largely unexplored. Here, we experimentally show that temperature strongly influences the emission of infochemicals by ladybeetle larvae, which in turn modifies the oviposition behaviour of conspecific females. Temperature also directly affects female perception of infochemicals and their oviposition behaviour. As discussed below, these temperature-mediated effects on chemical communication may influence important population processes and thus alter the stability and dynamics of ecological communities.

As reported in previous studies (Hemptinne *et al*., 2001; Magro *et al*., 2007), we found that *A. bipunctata* larval infochemicals are mainly long-chain hydrocarbons that are typically involved in insect chemical communication (Blomquist & Bagnères, 2010). Interestingly, the composition and quantity of infochemicals (i.e. hydrocarbons) synthesized and emitted by ladybeetle larvae were strongly affected by temperature (Fig. 1a, direct effect). Quantitative changes were more important than qualitative (i.e. chemical composition) changes: only five chemical compounds of 61 were not present in all treatments. The quantity of infochemicals produced was approximately twice as high at 25 °C than at 15 and 20 °C. This pattern is consistent with previous studies on plants, where temperature was found to increase the emission of volatile organic compounds (Peñuelas & Staudt, 2010). According to MTE, the emission rate of larval infochemicals increased exponentially with temperature, and the slope of this relationship (0.49 ± 0.17 eV) was close to the predicted value (0.65 eV). However, our analyses showed that a quadratic model best described the relationship between temperature and infochemical emission rate, indicating that temperature dependence of the infochemical emission rate is stronger than what was predicted by MTE. The difference between observation and prediction of the null model (i.e. MTE) suggests that the production of infochemicals does not perfectly scale with metabolism rate, and thus, additional mechanisms, such as active infochemical release, might contribute to explain this difference. One nonexclusive hypothesis is that larvae produce more hydrocarbons at higher temperature to reduce hydric stress – long-chain cuticular hydrocarbons are known to protect arthropods from desiccation (Gibbs, 2002). However, the mechanisms behind this pattern and how they may be modulated by other physiological processes, such as thermal acclimation, remain to be investigated in more detail. Overall, our results suggest that the effects of climate change on chemical information may be stronger than its effects on other biological and interaction rates, which generally conform to MTE (Brown *et al*., 2004; Burnside *et al*., 2014).

Our results also indicate that the effects of temperature on infochemical emission indirectly influence the behaviour of the receiver (Fig. 1a, indirect effects); ladybeetles refrained more to lay eggs and laid fewer eggs in the presence of larval infochemicals emitted at the highest temperature. Interestingly, the quantity of infochemicals emitted at different temperatures seems to correlate positively with the strength of oviposition inhibition (see Figs 3 and 4), which supports prior results showing that females rely on the amount of infochemicals to assess the risk of their eggs being eaten by conspecifics (Doumbia *et al*., 1998; Oliver *et al*., 2006). Given that enzymatic pathways for hydrocarbons production are similar for most arthropods (Blomquist & Bagnères, 2010) and that most insects can detect small changes in the composition or concentration of chemical cues (Cardé & Millar, 2004; Heil, 2008; Hansson, 2014), we expect the direct and indirect effects of temperature on chemical communication to significantly influence intra- and interspecific interactions in most ecosystems.

Finally, temperature directly affected the receiver's behavioural response to infochemicals (Fig. 1c). Ladybeetles avoided laying eggs in the presence of infochemicals at 15 and 20 °C, but not at 25 °C. In addition, the number of eggs laid was significantly lower in the presence of infochemicals, and this difference was not affected by temperature. These results are unexpected because risk of predation and cannibalism generally increase with temperature (Ponsonby & Copland, 1998; Sentis *et al*., 2012), which – contrary to our observations – should reinforce oviposition inhibition. Two not mutually exclusive mechanisms may be invoked to explain this result. First, as warming increases the rate of oocyte maturation (Papaj, 2000; Berger *et al*., 2008), females might become less selective because they produce more eggs. Second, many insects, including ladybeetles, are known to be less selective when they are constrained by time, that is when they get old (Papaj, 2000; Fréchette *et al*., 2004). As temperature significantly reduces ectotherm's lifespan (Brown *et al*., 2004), it can also decrease female selectivity by increasing time constraint.

Overall, we showed that temperature modulates chemical communication in ladybeetles and thus plays a crucial role in the assessment of oviposition site quality by females. Temperature influences the composition and quantity of infochemicals, and females detect these changes and adjust their oviposition behaviour accordingly (i.e. reproduce less). However, the direct effect of temperature on ladybeetle females leads to the opposite trend by decreasing oviposition inhibition in response to infochemicals. These results therefore indicate that the consequences of climate change on chemical-mediated interactions are complex, as they depend on the relative effects of temperature on infochemical emission and perception and on species' responses to infochemicals (Fig. 1a,c).

The next logical step would be to determine how these thermal effects may cascade up and affect the dynamics of ecological networks. Kéfi *et al.* (2012) proposed a simple and elegant framework for integrating nontrophic interactions (such as oviposition) into network models. The authors categorized nontrophic interactions based on their direct effects on processes included in traditional food-web models, which leads to three nontrophic functional groups: those that modify (1) feeding parameters, (2) nonfeeding parameters and (3) flows across system boundaries (e.g. immigration, emigration). In the present study, we found that warming elicits an increase of infochemical emission, which in turn strengthens oviposition inhibition. Such a pattern may also increase patch leaving (i.e. emigration) behaviour by competitors relying on infochemicals to assess patch quality (Almohamad *et al*., 2010). Following Kéfi *et al.* (2012), we classify these effects as nontrophic interactions affecting flows across system boundaries as they enhance immigration (i.e. oviposition) and reduce emigration (i.e. patch leaving). According to theoretical studies (Mccann *et al*., 1998; Neutel *et al*., 2007; Kéfi *et al*., 2012), these effects on migration flow are likely to weaken predator–prey interaction strength and thus increase the stability and persistence of ecological networks. However, in contrast to the indirect effect of temperature on oviposition behaviour described above, the direct effect of temperature on ladybeetle females decreased their oviposition inhibition in response to infochemicals. This thermal effect may thus enhance immigration and strengthen predator–prey interactions, which in turn leads to network instability. We therefore predict that the net effect of warming on community stability depends on the relative importance of its effects on infochemical emission and on behavioural responses by the different species involved.

Both climate change and chemical ecology have received considerable attention given their importance to the physiology and ecology of species. However, very few links have been established between these two fields of research, thereby leaving a substantial gap in our understanding of how climate change may affect chemical information and species' interactions. Here, we showed (1) that temperature exponentially increases the emission of infochemicals by ladybeetle larvae, (2) that conspecific females perceive such changes and adjust their oviposition behaviour accordingly and (3) that the oviposition response to infochemicals also depends on the temperature at which infochemicals are perceived. Furthermore, our results suggest that temperature effects on chemical communication may influence flows across system boundaries (i.e. immigration and emigration) and thus affect the dynamics and stability of ecological networks. We therefore argue that investigating the effects of temperature on chemical communication is a crucial step towards a better understanding of the functioning of ecological communities facing rapid environmental changes. In this respect, our study reveals new ways to incorporate chemical information into temperature-dependent models using the theoretical framework developed by Kéfi *et al.* (2012) and also improves our ability to predict climate change consequences for species' interactions and ecological networks.

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References

Almohamad R, Verheggen FJ, Francis F, Lognay G, Haubruge E (2010) Assessment of oviposition site quality by aphidophagous hoverflies: reaction to conspecific larvae. *Animal Behaviour*, **79**, 589–594.

Berger D, Walters R, Gotthard K (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology*, **22**, 523–529.

Blomquist GJ, Bagnères A-G (2010) *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press, Cambridge, UK.

Brosse S, Grenouillet G, Gevrey M, Khazraie K, Tudesque L (2011) Small-scale gold mining erodes fish assemblage structure in small neotropical streams. *Biodiversity and Conservation*, **20**, 1013–1026.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Burnside WR, Erhardt EB, Hammond ST, Brown JH (2014) Rates of biotic interactions scale predictably with temperature despite variation. *Oikos*, **123**, 1449–1456.

Cardé RT, Millar JG (2004) *Advances in Insect Chemical Ecology*. Cambridge University Press, Cambridge.

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Doumbia M, Hemptinne JL, Dixon AFG (1998) Assessment of patch quality by ladybirds: role of larval tracks. *Oecologia*, **113**, 197–202.

Englund G, Ohlund G, Hein CL, Diehl S (2011) Temperature dependence of the functional response. *Ecology Letters*, **14**, 914–921.

Fréchette B, Dixon AFG, Alauzet C, Hemptinne JL (2004) Age and experience influence patch assessment for oviposition by an insect predator. *Ecological Entomology*, **29**, 578–583.

Fussmann KE, Schwarzmüller F, Brose U, Jousset A, Rall BC (2014) Ecological stability in response to warming. *Nature Climate Change*, **4**, 206–210.

Gibbs AG (2002) Lipid melting and cuticular permeability: new insights into an old problem. *Journal of Insect Physiology*, **48**, 391–400.

Gilbert B, Tunney TD, Mccann KS *et al*. (2014) A bioenergetic framework for the temperature dependence of trophic interactions. *Ecology Letters*, **17**, 902–9014.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. *Science*, **293**, 2248–2251.

Hansson BS (2014) From organism to molecule and back–insect olfaction during 40 years. *Journal of Chemical Ecology*, **40**, 409–410.

Heil M (2008) Indirect defence via tritrophic interactions. *New Phytologist*, **178**, 41–61.

Hemptinne JL, Lognay G, Doumbia M, Dixon AFG (2001) Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Chemoecology*, **11**, 43–47.

Hodek I, Honěk A (1996) *Ecology of Coccinellidae*. Kluwer, Boston.

Holopainen JK, Himanen SJ, Poppy GM (2013) Climate change and its effects on the chemical ecology of insect parasitoids. In: *Chemical Ecology of Insect Parasitoids* (eds Wajnberg E, Colazza S), pp. 168–190. John Wiley & Sons, Oxford, UK.

Jalali MA, Tirry L, Arbab A, De Clercq P (2010) Temperature-dependent development of the two-spotted ladybeetle, *Adalia bipunctata*, on the green peach aphid, *Myzus persicae*, and a factitious food under constant temperatures. *Journal of Insect Science*, **10**, 1536–1552.

Johansson BG, Jones TM (2007) The role of chemical communication in mate choice. *Biological Reviews*, **82**, 265–289.

Kéfi S, Berlow EL, Wieters EA *et al*. (2012) More than a meal... integrating non-feeding interactions into food webs. *Ecology Letters*, **15**, 291–300.

Magro A, Téné JN, Bastin N, Dixon AFG, Hemptinne JL (2007) Assessment of patch quality by ladybirds: relative response to conspecific and heterospecific larval tracks a consequence of habitat similarity? *Chemoecology*, **17**, 37–45.

Martini X, Haccou P, Olivieri I, Hemptinne J (2009) Evolution of cannibalism and female's response to oviposition-deterring pheromone in aphidophagous predators. *Journal of Animal Ecology*, **78**, 964–972.

Mcardle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82**, 290–297.

Mccann KS, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. *Nature*, **395**, 794–798.

Mcneil JN (1991) Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Annual Review of Entomology*, **36**, 407–430.

Millar JG, Sims JJ (1998) Preparation, cleanup, and preliminary fractionation of extracts. In: *Methods in Chemical Ecology* (eds Millar JG, Haynes KF), pp. 1–37. Kluwer, New-York.

Mondor E, Ay MT, CaW Mack, Lindroth RL (2004) Divergent pheromone-mediated insect behaviour under global atmospheric change. *Global Change Biology*, **10**, 1820–1824.

Neutel AM, Heesterbeek JaP, Van De Koppel J *et al*. (2007) Reconciling complexity with stability in naturally assembling food webs. *Nature*, **449**, 599–602.

Oksanen J, Kindt R, Legendre P, O'hara B, Simpson GL, Stevens MHH, Wagner HH (2008) Vegan: Community Ecology Package. R package version 1.13-1. http. vegan. r-forge. r-project. org/i.

Oliver TH, Timms JEL, Taylor A, Leather SR (2006) Oviposition responses to patch quality in the larch ladybird *Aphidecta obliterata* (Coleoptera: Coccinellidae): effects of aphid density, and con- and heterospecific tracks. *Bulletin of Entomological Research*, **96**, 25–34.

Papaj DR (2000) Ovarian dynamics and host use. *Annual Review of Entomology*, **45**, 423–448.

Peñuelas J, Staudt M (2010) BVOCs and global change. *Trends in Plant Science*, **15**, 133–144.

Pereira HM, Leadley PW, Proença V *et al*. (2010) Scenarios for global biodiversity in the 21st century. *Science*, **330**, 1496–1501.

Ponsonby D, Copland M (1998) Environmental influences on fecundity, egg viability and egg cannibalism in the scale insect predator, *Chilocorus nigritus*. *BioControl*, **43**, 39–52.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Seagraves M (2009) Lady beetle oviposition behavior in response to the trophic environment. *Biological Control*, **51**, 313–322.

Sentis A, Hemptinne JL, Brodeur J (2012) Using functional response modeling to investigate the effect of temperature on predator feeding rate and energetic efficiency. *Oecologia*, **169**, 1117–1125.

Sentis A, Hemptinne JL, Brodeur J (2013a) Effects of simulated heat waves on an experimental plant–herbivore–predator food chain. *Global Change Biology*, **19**, 833–842.

Sentis A, Hemptinne JL, Brodeur J (2013b) Parsing handling time into its components: implications for responses to a temperature gradient. *Ecology*, **94**, 1675–1680.

Sentis A, Hemptinne J-L, Brodeur J (2014) Towards a mechanistic understanding of temperature and enrichment effects on species interaction strength, omnivory and food-web structure. *Ecology Letters*, **17**, 785–793.

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.

Van Damme R, Bauwens D, Vanderstighelen D, Verheyen RF (1990) Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature. *Animal Behaviour*, **40**, 298–305.

Yuan JS, Himanen SJ, Holopainen JK, Chen F, Stewart CN Jr (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. *Trends in Ecology & Evolution*, **24**, 323–331.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Methods for chemical analyses.
Table S1. Qualitative and quantitative analyses of the infochemicals emitted by *Adalia bipunctata* larvae at 15, 20, and 25 °C.
Fig. S1. Chromatograms of *Adalia bipunctata* larval infochemicals at 15, 20, and 25 °C obtained using a CG-MS.
Fig. S2. Non-metric multidimensional scaling ordinations (NMDS) for the (a) qualitative and (b) quantitative composition of *Adalia bipunctata* larval infochemicals at 15, 20, and 25 °C.