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SHORT COMMUNICATION

High temperature and temperature variation undermine future disease susceptibility in a population of the invasive garden ant *Lasius neglectus*

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Abstract Environmental temperature and temperature variation can have strong effects on the outcome of host-parasite interactions. Whilst such effects have been reported for different host systems, long-term consequences of pre-infection temperatures on host susceptibility and immunity remain understudied. Here, we show that experiencing both a biologically relevant increase in temperature and temperature variation undermines future disease susceptibility of the invasive garden ant *Lasius neglectus* when challenged with a pathogen under a constant temperature regime. In light of the economic and ecological importance of many social insects, our results emphasise the necessity to take the hosts' temperature history into account when studying host-parasite interactions under both natural and laboratory conditions, especially in the face of global change.

Keywords Ecological immunology · Climate change · Host-parasite interaction · Entomopathogenic fungi · Immunity · Acclimation

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Introduction

Most invertebrates are ectothermic, and consequently environmental temperature plays an important role in their physiology, behaviour and ultimately fitness. Host-parasite interactions are no exception to this, and several studies have shown that environmental temperature can severely affect the outcome of host-parasite interactions, with temperature influencing invertebrate resistance and immunity as well as parasite virulence and growth (Adamo and Lovett 2011; Linder et al. 2008; Thomas and Blanford 2003). In contrast to what is often assumed in such studies, environmental parameters are rarely constant and variation in temperature and other environmental aspects influence the outcome of host-parasite interactions as well (Fischer et al. 2011; Murdock et al. 2013; Triggs and Knell 2012). Apart from immediate effects of environmental temperature and temperature variation, the temperature experienced before infection can also have longer lasting effects on the outcome of future host-parasite interactions. For example, fruit flies, *Drosophila melanogaster*, have a higher resistance against bacteria and a higher expression of an antimicrobial peptide when they experienced low temperature regimes for 24 h before infection (Linder et al. 2008). Recently, also trans-generational effects on immunity and susceptibility were found upon parental temperature shock treatment (Eggert et al. 2015).

Long-term effects of pre-infection temperatures on the outcome of future host-parasite interactions have so far received little attention. Here, we investigate how pre-infection exposure to different, biological relevant, temperatures and temperature variation regimes affects parasite defence and susceptibility of the invasive garden ant *Lasius neglectus* when exposed to the generalist entomopathogenic fungus *Beauveria bassiana*. Theory predicts that social insects might be particularly susceptible to the spread of parasites and diseases as their nests are often characterised by high densities of genetically similar individuals (Schmid-Hempel 1998). To counter these risks, social insects have evolved effective external and behavioural immune defence traits that can be socially mediated and complement internal immune defence mechanism (Cremer et al. 2007; Otti et al. 2015). How these defence mechanisms and the susceptibility to parasites are affected by previously experienced temperature is largely unknown.

Materials and methods

We used the invasive garden ant *Lasius neglectus* and the entomopathogenic fungus *Beauveria bassiana* as a study system (for details see ESM 1).

Experimental set-up

We randomly assigned 36 experimental nests containing 25 workers and 3–6 larvae (total no. workers = 900) to three constant temperature regimes (15, 20 and 25 °C) and one that varied between 15 and 25 °C at a 24-h interval resulting in a mean temperature of 20 °C (no. workers per temperature regime = 225) for 22 days. After this period, all nests were kept at a constant temperature of 20 °C, to measure the influence of pre-infection temperature on future disease susceptibility under constant temperature. On day 25, we sampled four workers from all nests and immediately stored them at −28 °C for later quantification of the ants' ability to inhibit fungal growth (non-exposed individuals). On day 26, we individually exposed all remaining ants by topical application of *B. bassiana* (0.5 μl 1×10^9 spores/ml). On day 27, we again randomly sampled four workers per nest to measure their ability to inhibit fungal growth (exposed individuals). Survival was monitored over the whole experimental period. For further details, see ESM 1.

Fungal growth inhibition

To estimate the ants' ability to inhibit fungal growth, pooled exposed and non-exposed ants per nest stored at −28 °C were ground in liquid nitrogen and re-suspended in 5 μl 0.05 % Triton X-100 (TX) per ant. Five microlitre of this ant solution was mixed with 95 μl of fungal conidiospore suspension (1×10^3 spores/ml) and 100 μl of the resulting mixture plated on malt extract agar plates. As a control, we used 95 μl fungal conidiospore suspension mixed with 5 μl pure 0.05 % TX. The plates were incubated for 3 days at 24 °C, and the number of growing fungal colonies was counted.

Sanitary grooming behaviour

We filmed all nests (Logitech C910) for 3 min on the day before starting the temperature regimes (day 0), 1 day after finishing the temperature regimes (day 23) and 4 h after challenging the ants with the fungus (day 26) to quantify an influence of temperature regimes on grooming behaviour. For each 3-min video, we performed scan samples at 10-s intervals (18 scans per video) and recorded the number of ant workers performing self- and allogrooming.

Statistical analysis

Survival of worker ants during temperature regimes (days 1–22) and after pathogen exposure (days 26–38) was analysed separately with Cox mixed-effects models including experimental nest as a random factor. The ability to inhibit fungal growth of non-exposed (day 25) and exposed (day 27) ants was analysed using one-way ANOVAs. Grooming behaviour was analysed in a Linear Mixed Model (LMM) including the type of temperature regime, the time grooming behaviour was observed and their interaction as fixed effects and time of measurement given nest as a random effect (see also ESM 1).

Results

The different temperature regimes during the 22 days prior to pathogen exposure did not influence *Lasius neglectus* survival (ESM Fig. 1; Cox mixed-effects model: Wald—$\chi^2_3 = 5.927$, $P = 0.115$). However, following pathogen exposure, the previously experienced temperature regimes significantly affected survival probability under the now identical temperature

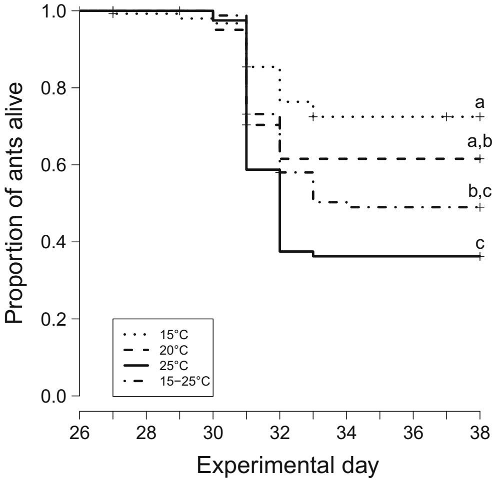

Fig. 1 Proportion of ants that survived after exposure to the fungus *Beauveria bassiana* under constant 20 °C. Prior to fungal exposure, ants were kept either at 15 °C (*dotted line*), 20 °C (*dashed line*) or 25 °C (*solid line*) constant temperatures or one varying temperature between 15–25 °C (*dotted-dashed line*)

condition across treatment groups (Fig. 1; Wald—$\chi^2_3 = 21.65$, $P < 0.001$). Survival deteriorated from ants that had previously experienced low (15 °C) to ants that had experienced high (25 °C) temperatures with ants having experienced temperatures 15–25 °C lying between the survival of ants kept at 20 and 25 °C (Tukey post hoc tests: 15 °C vs. cycling 15–25 °C: $P = 0.017$, 25 vs. 15 °C: $P < 0.001$, 25 vs. 20 °C: $P = 0.009$, all other comparisons: $P > 0.05$).

The ability of ants to inhibit fungal growth was affected by the experienced temperature regimes (Fig. 2a; non-exposed ants on day 25; ANOVA: $F_{4,37} = 4.263$, $P = 0.006$) but not by pathogen challenge under constant temperature conditions (ESM Fig. 2; exposed ants on day 27; ANOVA: $F_{4,40} = 1.340$, $P = 0.272$). Interestingly, the ability to inhibit fungal growth prior to pathogen exposure matched survival patterns after pathogen exposure. Non-exposed ants that had experienced 15 or 20 °C significantly inhibited fungal growth compared to ants that had experienced 25 or 15–25 °C (Fig. 2a; Tukey post hoc tests: 15 or 20 vs. 25 or 15–25 °C: $P < 0.04$, all other pairwise comparisons: $P > 0.1$).

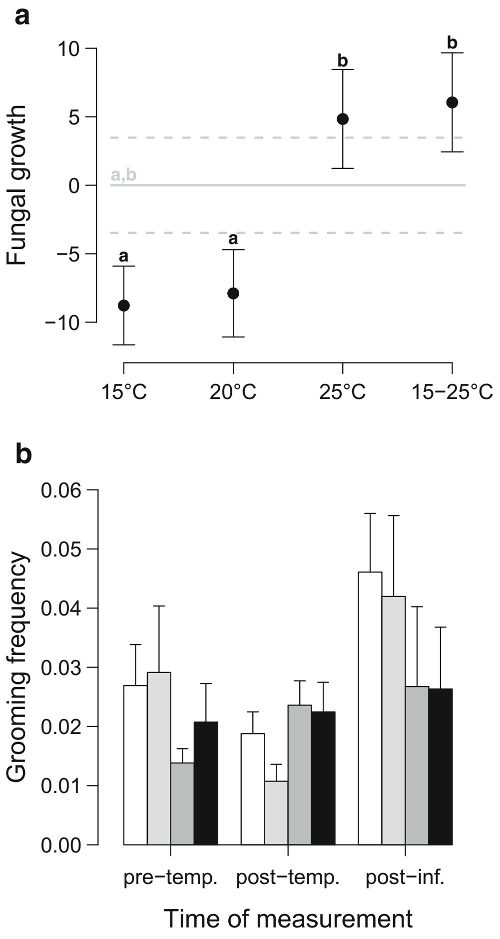

Fig. 2 **a** Fungal growth inhibition of non-exposed ants (mean ± s.e.) relative to control (mean ± s.e. in *solid* and *dashed grey lines*, respectively) on day 25 under constant 20 °C after experiencing different temperature regimes for 22 days. *Letters* indicate statistical significant differences for $\alpha = 0.05$. **b** Frequency of grooming behaviour (mean ± s.e.) before temperature regimes (day 0, pre-temp.), after temperature regimes (day 23, post-temp.) and after fungal exposure (day 26, post-inf.) for ants that were kept at 15 °C (*white bars*), 20 °C (*light grey bars*), 25 °C (*dark grey bars*) and 15–20 °C (*black bars*) during days 1–22

In contrast to the ability to inhibit fungal growth, grooming behaviour was not affected by temperature regimes, time of measurement or an interaction between both (LMM; LR test; temperature regimes × time of measurement: $\chi^2_6 = 8.608$, $P = 0.197$; temperature regimes: $\chi^2_3 = 1.164$, $P = 0.762$; time of measurement: $\chi^2_2 = 3.779$, $P = 0.151$). However, grooming frequency after pathogen exposure was 2.4 and 3.8 times higher compared to before exposure in ants that had experienced 15 and 20 °C, respectively, whilst ants that had experienced 25 °C and varying temperatures showed only a 1.1 and 1.2 times higher grooming frequency after exposure compared to before, respectively (Fig. 2b, ESM Table 1).

Discussion

In order to gain a better understanding of how changes in environmental temperature relate to social insect health and parasite susceptibility, we infected workers of the ant *Lasius neglectus* with the entomopathogenic fungus *Beauveria bassiana* following exposure to different pre-infection temperature and temperature variation regimes. We show that *Lasius neglectus* workers are more susceptible to *Beauveria bassiana* under constant temperature conditions if the ant prior to infection had experienced a period of high or varying temperature conditions. Moreover, our results indicate that this effect is potentially caused by a combination of a reduced ability to inhibit fungal growth and maybe also by an inability of ants to adequately adjust grooming behaviour. The validity of our findings will have to be corroborated further by using different temperature regimes and different *Lasius neglectus* populations, as there is the possibility that *Lasius neglectus* has adapted to different local temperatures in its invasive range, similar to the invasive ant *Wasmannia auropunctata* (Rey et al. 2012).

Strong effects of temperature and temperature variation on the expression of immune traits have been documented in previous studies (Adamo and Lovett 2011; Catalán et al. 2012; Murdock et al. 2013; Triggs and Knell 2012). Traditionally, variation in the outcome of host-parasite interactions at different temperatures has been interpreted as a result of variation in host and parasite temperature optimums (Lazzaro and Little 2009; Thomas and Blanford 2003). As ant hosts in our experiment experienced temperature regimes before exposure to *Beauveria bassiana*, it is unlikely that our results can be explained by different temperature optimums of the host and the parasite. One explanation of our results might be the activation of a stress response caused by increases in temperature and temperature variation. The activation of stress responses due to temperature regimes has been found in previous studies (Fischer et al. 2011; Linder et al. 2008). Both short-term and long-term activation of a stress response can negatively impact immune function and behaviour in animals across phyla (Adamo 2014; Roeder 2005).

The success of invasive ant species is affected by many biotic and abiotic factors (Krushelnycky et al. 2009). Climatic suitability has emerged as the most relevant variable in shaping the worldwide distribution of the invasive Argentine ant (Roura-Pascual et al. 2011). The results obtained in this study suggest a potential of host-parasite interactions to contribute limiting the future invasive spread of *Lasius neglectus* in European areas most affected by increasing temperatures due to global change. This will however strongly depend on the type of host-parasite interactions encountered in the field and on the influence of temperature on parasite virulence and growth.

Overall, our results highlight the importance of temperature and temperature variation when studying host-parasite interactions in the context of both short-term (season) and long-term (global warming) temperature variation. In this respect, social insects are an important study as, on one hand, important ecosystem services are provided by social insects and potentially threatened by climate change and novel host-parasite interactions due to climate change, e.g. in bees (Goulson et al. 2015) and on the other hand climate change might favour social insect and parasite invaders (Goulson et al. 2015; Walther et al. 2009).

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