

# Thermal biology of the meadow grasshopper, *Chorthippus parallelus*, and the implications for resistance to disease

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**Abstract.** 1. The thermal biology of the meadow grasshopper, *Chorthippus parallelus*, a common, habitat generalist acridid species found in the U.K., was characterised and the influence of thermoregulatory behaviour for resistance against a temperate (*Beauveria bassiana*) and tropical (*Metarhizium anisopliae* var. *acridum*) fungal pathogen was determined.

2. *Chorthippus parallelus* was found to be an active behavioural thermoregulator, with a preferred temperature range of 32–35 °C.

3. Both pathogens proved lethal to fifth instar and adult grasshoppers. No evidence of behavioural fever in response to infection by either pathogen was found, but normal thermoregulation was found to reduce virulence and spore production of *B. bassiana*. Normal thermoregulation did not appear to affect *M. anisopliae* var. *acridum*.

4. These results suggest that the effects of temperature on host resistance depend on the thermal sensitivity of the pathogen and, in this case, derive from direct effects of temperature on pathogen growth rather than indirect effects mediated by host immune response.

5. The implications for possible risks of exotic pathogens and influence of climate change are discussed.

**Key words.** *Beauveria bassiana*, behavioural fever, behavioural thermoregulation, *Chorthippus parallelus*, climate change, *Metarhizium anisopliae* var. *acridum*.

## Introduction

Many Orthoptera species, in particular many members of the Acrididae, have been shown to be active behavioural thermoregulators (Uvarov, 1966; Willott, 1997; Klass, 2004). Through a combination of selection of microclimates within a habitat and body orientation relative to solar radiation and air movements, these insects seek to achieve their preferred body temperatures, which may differ markedly from the ambient temperature. Body temperature preferences and thermoregulatory ability vary between species relative to climate and habitat composition (May, 1985).

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The ability of pathogens to kill ectotherm hosts has been shown to depend on host body temperature and how this fluctuates with environmental conditions (see review in Thomas & Blanford, 2003). A substantial body of work has been published concerning the role of host thermal biology in modulating host–pathogen interactions in the Orthoptera. This has focused mainly on the implications for using fungal pathogens, such as *Metarhizium anisopliae* var. *acridum* (formerly *Metarhizium flavoviride* Gams and Rozsypal, Driver *et al.*, 2000) and *Beauveria bassiana* (Balsamo) Vuillemin, as biological pest control agents of economically damaging species in semiarid regions.

The optimal temperatures for growth (i.e. where growth is = 60% of the maximum growth rate) of *B. bassiana* are in the range of 20–30 °C, with an upper limit of ≈36 °C (Fargues *et al.*, 1997) and for *M. anisopliae* var. *acridum* between 20 and 35 °C, with an upper limit of ≈40 °C (Thomas & Jenkins, 1997). Many members of the

Acrididae (particularly those from semiarid regions) exhibit thermoregulatory behaviour enabling them to raise their temperatures to a preferred set point around 38–40 °C (Carruthers *et al.*, 1992; Inglis *et al.*, 1997; Blanford *et al.*, 1998; Blanford & Thomas, 1999a, b). Consequently, when infected with these pathogens, the opportunity to thermoregulate has been shown to increase survival time (Inglis *et al.*, 1997; Blanford & Thomas, 1999b); Ouedraogo *et al.*, 2002). In addition to normal thermoregulation, many species have been found to elicit a 'behavioural fever' response to infection, raising their preferred body temperature several degrees higher than normal (Inglis *et al.*, 1996; Adamo, 1998; Blanford & Thomas, 1999a, b; Elliot *et al.*, 2002; Bunday *et al.*, 2003). This behavioural fever has been shown to provide additional survival benefits (Elliot *et al.*, 2002), although the extent to which the effects of raising body temperature on an infection are due to the parasite thermal sensitivity or to an increased immune response in the host is unclear (Thomas & Blanford, 2003).

Behavioural thermoregulation has been shown for a number of British Orthoptera species (Begon, 1983; Willott, 1997). The temperature preferences and extent of thermoregulatory ability exhibited by these species are a determinant in habitat partitioning and geographic distribution (Willott & Hassall, 1998). However, to the best of our knowledge, the role of thermoregulation in disease resistance and possible behavioural changes in response to pathogens, have not been studied for U.K. species. The purpose of this study therefore was to investigate the thermal biology of the meadow grasshopper, *Chorthippus parallelus* Zetterstedt (Orthoptera: Acrididae), a widespread British acridid species occurring in a range of coarse grassland habitats, and the implications of thermal behaviour for disease resistance. The approach used in this study involved a combination of field and laboratory investigations to determine the thermal preference of *C. parallelus*, coupled with laboratory experiments to investigate the role of thermoregulation (including possible behavioural fever responses) for resistance against two mitosporic fungal pathogens (*M. anisopliae* var. *acridum* and *B. bassiana*) which differed in their thermal performance profiles.

## Methods

### *Thermal biology in the field*

A number of study sites with a variety of sward heights were identified around the village of Wye (U.K.) where *C. parallelus* was present. Individual insects were captured using a sweep net or by hand. Body temperatures were measured following the method of Blanford *et al.* (1998); a small hole was made in the thorax using the tip of a 0.22 mm diameter hypodermic needle and a 0.125-mm diameter copper–constantin thermocouple connected to a digital thermometer inserted to a depth of 2 mm. The thermocouple was connected to a hand-held digital thermometer. Body temperature readings were taken once they

had stabilised and within 8 s of capture. After 8 s, handling may begin to affect body temperature so recordings exceeding this time limit were discarded. Only adult or fifth instar insects were used, as the thermal biology of acridids differs between these and earlier life cycle stages (Uvarov, 1966).

Sampling was carried out over a number of days during July and August 2004, with individuals sampled throughout different hours of the day and night. Measurements of ambient temperatures were recorded at the soil surface and at 1, 15, and 40 cm heights in vegetation at each site, concurrent with sampling. Ambient data were collected at 1-min intervals using thermocouple probes attached to a 1000 series Squirrel datalogger (Grant Instruments, Cambridge, U.K.). These data were averaged to give mean ambient temperatures for each 10-min interval.

A body temperature model was generated for daylight hours by plotting the relationship between ambient temperature ( $T_a$ ) and body temperature ( $T_b$ ). Non-linear regression was carried out using the sigmoid function proposed by Samietz and Köhler (1998) (cited in Klass, 2004):

$$T_b = \left[ \frac{T_{\max} - T_a}{1 + \left( \frac{T_a}{T_{\text{inf}}} \right)^s} \right] + T_a$$

where  $T_{\max}$  is the asymptotic maximum body temperature;  $T_{\text{inf}}$  is the ambient temperature at the inflection point of the sigmoid curve and  $s$  is the slope parameter.  $T_{\max}$ ,  $T_{\text{inf}}$ , and  $s$  are parameter constants, which were estimated through standard non-linear procedures (Levenberg–Marquardt Estimation Method) in SPSS for Windows v.11.5 (SPSS Inc., 2003). This function has proved more accurate in predicting body temperatures at high and low temperatures than linear, logistic and polynomial methods (Klass, 2004). Moreover, addition of other environmental variables, such as wind speed and/or solar radiation, tend to improve model fit only marginally (Kemp, 1986; Klass, 2004).

### *Assessment of preferred body temperatures using thermal gradients in the laboratory*

Field-caught fifth instar and adult *C. parallelus* were placed in 12 cages (35 × 35 × 44 cm), with 15 insects per cage. Cages were aluminium sided with perforated floors to prevent accumulation of frass. Cages were maintained at a constant temperature (20 °C) in controlled temperature rooms with a photoperiod of LD 16:8 h. Insects were provided with freshly cut wheat seedlings every 2 days as food.

These life stages are of similar size and all insects were collected from a brachypterous population (which is the norm for adults of this species) and selected randomly for experimental treatments. In studies of other acridid species, no difference in thermoregulatory preference and ability has been found between late instars and adults (Chapman, 1965; Uvarov, 1966; Blanford & Thomas, 1999a).

Oil formulations of *B. bassiana* (I93-825) and *M. anisopliae* var. *acridum* (IMI330189) were prepared, with spore concentrations adjusted to give  $1 \times 10^4 \mu\text{l}^{-1}$

using an improved Neubauer haemocytometer. *Beauveria bassiana* I93-825 is a temperate pathogen from North America and *M. anisopliae* var. *acridum* IMI330189 is a tropical pathogen originating in arid/semiarid areas of Niger. Isolates of both of these fungal species have been found during soil sampling within the U.K. (Chandler *et al.*, 1997) although their host range and virulence remain largely undetermined.

In a number of previous studies, pathogens were administered to locusts/grasshoppers by application of oil droplets containing conidia to the base of the pronotum (Blanford *et al.*, 1998; Blanford & Thomas, 1999b, 2000; Elliot *et al.*, 2002; Klass, 2004). This method was attempted with *C. parallelus* using different proportions of Ondina and Shellsol oils in the formulation but control mortality was unacceptably high. To infect insects in the current study therefore oil formulations were applied to 10 insects at a time in 400-ml plastic containers, using an airbrush spray gun (Model 250, Badger Products, Franklin Park, IL). A 1-s spray burst was estimated to reliably deliver approximately 10 µl into the container. Following the initial burst, insects remained within the container for 10 s to allow coverage by the resulting vapour. This resulted in an even, thin covering of oil on all insects that caused no immediate mortality. Insects from four cages were sprayed with *B. bassiana*, four with *M. anisopliae* var. *acridum*, and four with blank oil (1.25% Shellsol : 98.75% Ondina) as controls. All cages were maintained at a constant 20 °C throughout. Two cages from each spray treatment were used to supply insects for thermal gradient studies (see below).

An aluminium thermal gradient (1 m long, 8 cm wide, 4 cm deep) was set up with one end resting in an ice bath and the other placed in a controlled hot water bath. This created a gradient from 10 to 45 °C. A clear acetate lid was placed on top with holes drilled every 2.5 cm to allow air circulation and insertion of thermocouples. Five insects were collected from a cage and introduced to the middle of the gradient and left for 30 min. The temperature at the position of each insect was then recorded, with subsequent recordings every 15 min for 1.5 h (i.e. seven recording episodes). Wire thermocouple tips connected to a digital thermometer were placed as close to the midpoint of the thorax as possible without making contact. After 2 h in the gradient, insects were removed and placed back in their original cage. This process was then repeated for the other gradient cages, giving a total of 10 insects and 70 thermal measurements for each spray treatment per day. This was repeated daily from days 2 to 10 after spraying. Gradients were cleaned with 70% ethanol after each run. The overall methodology follows that of numerous previous studies (e.g. Inglis *et al.*, 1996; Blanford & Thomas, 1999b).

In addition to the examination of thermal behaviour, which was the primary aim of the experiment, mortality was recorded daily up to 11 days after spraying and cadavers immediately removed. These were allowed to dry in Petri dishes for several days to reduce bacterial decay and then incubated at 25 °C on moistened filter paper to encourage sporulation. Mean survival time for each treatment was determined using Kaplan–Meier survival analysis in

SPSS for Windows v.11.5 (SPSS Inc., 2003). Differences in mean survival times between treatments were tested for significance using a log rank test.

#### *Effects of thermoregulation on resistance to disease*

Field-caught fifth instar and adult *C. parallelus* were placed in 18 cages (as above), with 20 insects per cage. Grasshoppers were maintained at a constant temperature (20 °C) with a photoperiod of LD 16:8 h and fed as above.

Insects were treated with *B. bassiana*, *M. anisopliae* var. *acridum*, or blank oil control as in the previous experiment, with six cages per treatment. Three cages from each spray treatment contained a 60-W light bulb and a 'climbing frame', providing a range of opportunities for thermoregulation during the day, from room temperature up to 45 °C. Bulbs were illuminated for 6.5 h during the middle of the daylight period (derived from rounded average of mean daily sunshine hours for July and August, Wye meteorological station, 1971–2000). The remaining cages were also equipped with a climbing frame but contained no light bulb and so provided no opportunity for insects to behaviourally thermoregulate. Relative humidity was not controlled for, as this has been shown not to affect the development of infections in oil-inoculated insects (Bateman *et al.*, 1993). After 40 days, light bulbs were turned off and ambient temperature set at 25 °C, in order to determine pathogen survival in any remaining insects.

As before, mortality was recorded daily, with cadavers removed and incubated to test for sporulation. Mean survival times for the different treatments were determined as above.

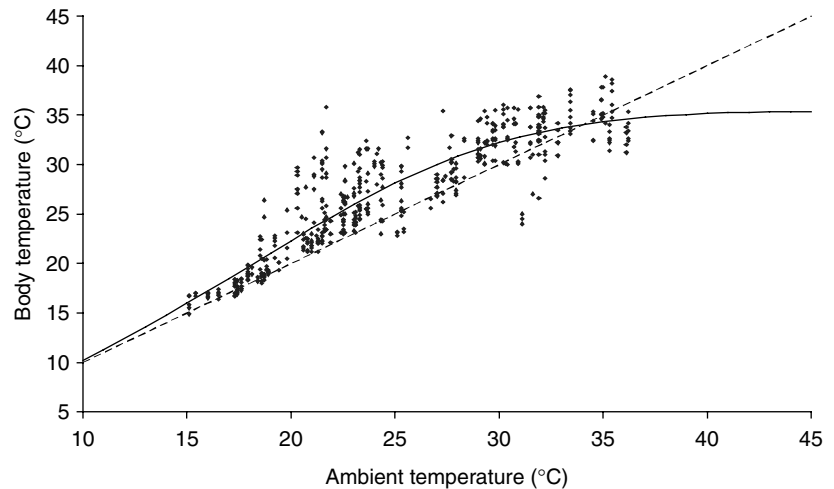
## Results

### *Thermal biology in the field*

Ambient temperatures varied with weather conditions and habitat characteristics. The measured maximum and minimum temperatures in daylight hours were 15.1 °C and 36.2 °C, respectively. Night-time temperatures were consistently below 20 °C. Weather station data for July gave a daily maximum of 27.0 °C and a minimum of 6.3 °C. For August these figures were 29.6 °C and 13.4 °C, respectively.

Late instar and adult *C. parallelus* were found in all but the longest, densest grass swards. Body temperatures recorded during the hours of darkness remained close to ambient. During hours of daylight, however, body temperatures frequently exceeded ambient with a mean elevation above ambient of  $2 \pm 0.12$  °C, with a range from  $-7.1$  to  $+14.1$  °C. The highest body temperature observed was 38.9 °C and the lowest 14.9 °C (Fig. 1). The greatest elevations above ambient were observed when direct sunlight first became available in the morning. The non-linear regression of body temperature against ambient indicated a preferred body temperature ( $T_{max}$  in the sigmoid model) of approximately 34 °C.

**Fig. 1.** Distribution of body temperatures ( $T_b$ ) against ambient temperatures ( $T_a$ ) for *Chorthippus parallelus*. The broken line shows a null model where  $T_b = T_a$ . The solid line represents the best-fit regression curve based on the sigmoid body temperature model. The intersection of the regression curve with the null model indicates preferred body temperature. Parameter estimates (with asymptotic standard errors) and  $r^2$  for the sigmoid model:  $T_{\max} = 34.04$  °C ( $\pm 0.382$ );  $T_{\text{infl}} = 28.83$  °C ( $\pm 1.175$ );  $s = -4.48$  ( $\pm 0.538$ );  $r^2 = 0.82$ .  $n = 500$ .



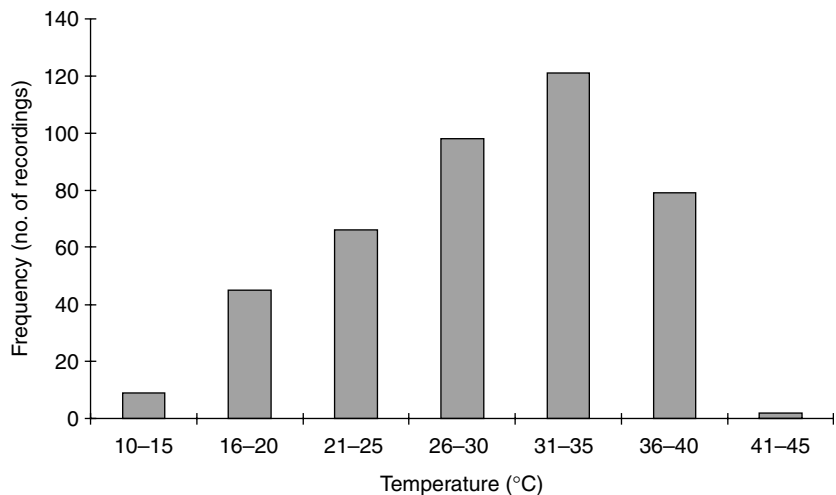
#### Assessment of preferred body temperatures using thermal gradients in the laboratory

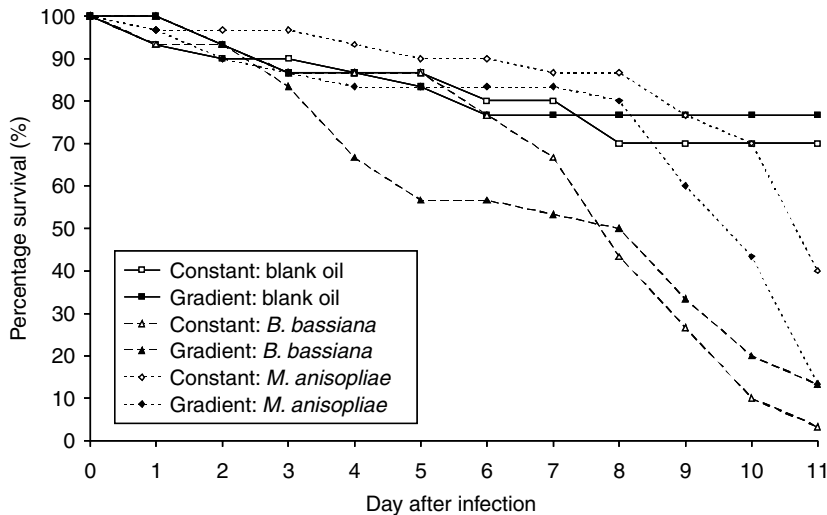
The thermal gradient studies showed that the preferred temperature selected on the gradient by *C. parallelus* was in the range 31–35 °C (Fig. 2), with a modal temperature for the blank oil control treatment of 32.8 °C (data pooled over days 2–7;  $n = 420$ ). No significant difference was found between the blank oil and the *B. bassiana* treatment ( $F_{1,838} = 0.73$ ,  $P = 0.393$ ,  $n = 420$ ), or the *M. anisopliae* var. *acridum* treatment ( $F_{1,838} = 2.19$ ,  $P = 0.139$ ,  $n = 420$ ) for this time period. By day 9, the pattern had changed with both pathogen treatments highly significantly different to the control (*B. bassiana*,  $F_{1,138} = 35.25$ ,  $P < 0.001$ ,  $n = 560$ ; *M. anisopliae* var. *acridum*,  $F_{1,138} = 45.45$ ,  $P < 0.001$ ,  $n = 560$ ). These differences were represented by a lower temperature distribution in infected insects. Whilst this could be indicative of an afebrile response, the advanced mycosis at this time meant that individuals were much less mobile when placed on the gradient and generally remained in the position at which they were

introduced. Overall, aside from minimal spatial displacement when insects attempted to occupy the same point in the gradient, no evidence of behavioural interactions between insects was apparent and individuals were observed to control their own temperature freely by movement within the gradient. On the basis of this evidence, there appears to be no alteration in thermoregulatory response to infection with these pathogens (i.e. no evidence of behavioural fever, or afebrile responses).

Control insects sprayed with blank oil showed little mortality over the course of the experiment, with no significant difference in mean survival time between those held at constant temperature and those exposed to the gradient (mean survival time  $\pm$  SE of  $9.17 \pm 0.58$  and  $9.33 \pm 0.57$  days, respectively; log rank statistic = 0.25;  $P = 0.62$ ) (Fig. 3). Mean survival times of the pathogen-treated populations were generally significantly less than relevant controls (*B. bassiana*-constant  $7.70 \pm 0.49$  days, log rank statistic = 21.26,  $P < 0.001$ ; *B. bassiana*-gradient  $7.13 \pm 0.60$  days, log rank statistic = 20.00,  $P < 0.001$ ; *M. anisopliae* var. *acridum*-gradient  $8.90 \pm 0.57$  days, log

**Fig. 2.** Frequency distributions at 5 °C intervals of positions selected by *Chorthippus parallelus* treated with blank oil and placed on a thermal gradient. Columns represent pooled data from days 2 to 7 post exposure ( $n = 420$ ).





**Fig. 3.** Cumulative percentage survival of *Chorthippus parallelus* exposed to blank oil and pathogen treatments under different thermal regimes: Constant, constant temperature (20 °C); Gradient, 2 h on thermal gradient (10–45 °C) for random sub-sample of insects each day. For all treatments,  $n = 30$ .

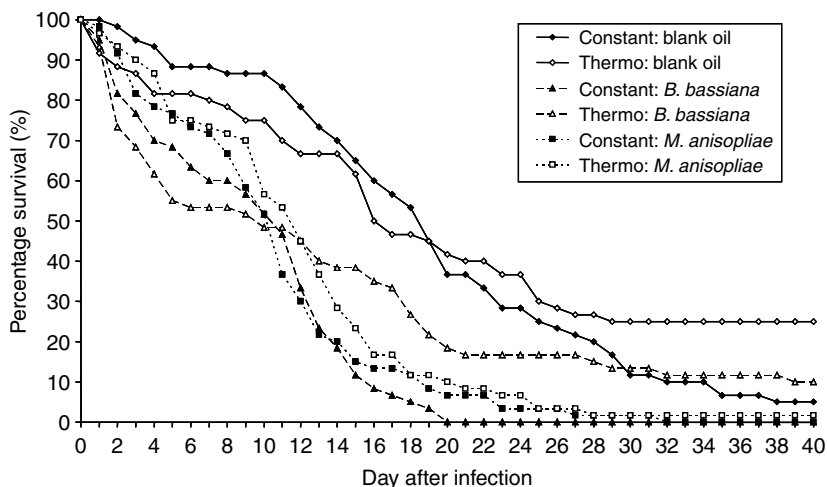
rank statistic = 16.92,  $P < 0.001$ ). The exception was the constant temperature *M. anisopliae* var. *acridum* treatment, which did not differ from the constant temperature control (mean survival time  $9.83 \pm 0.45$ , log rank statistic = 2.75,  $P = 0.971$ ). This is most likely due to the late onset of mortality in this pathogen treatment and the fact that populations were censored on day 11. However, this meant short-term exposure to the gradient did alter survival between *M. anisopliae* var. *acridum* treatments (log rank statistic = 5.96,  $P = 0.02$ ). No equivalent effect was seen with *B. bassiana* (log rank statistic = 0.29,  $P = 0.59$ ).

Aside from normal saprotrophic fungi, no sporulation was observed on cadavers from either blank oil treatment. Percentage sporulation in *B. bassiana* treatments was 58.6% for the non-gradient cages and 24.0% for the gradient cages ( $G$ -test = 49.48,  $P < 0.001$ ). For the *M. anisopliae* var. *acridum* treatments, figures were 50.0% for the non-gradient cages and 46.2% for the gradient cages ( $G$ -test = 0.578,  $P = 0.447$ ). No sporulation of the test pathogens was observed in insects dying before day 6, although two unidentified fungi were observed on infected

cadavers from day 2. Whether these were pathogenic species is unknown at this time. A parasitoid fly larva (Family Sarcophagidae) emerged from one gradient control insect several days after death. No other evidence of parasitism by insects was observed in any other treatments.

#### Effects of thermoregulation on resistance to disease

For control insects treated with blank oil only, there was no significant effect of thermoregulation on mean survival time by the end of the study (mean survival times of  $19.50 \pm 1.24$  and  $20.22 \pm 1.74$  days for constant and thermoregulation treatments, respectively; log rank statistic = 1.86,  $P = 0.172$ ), although the percentage of survivors at day 40 was significantly higher under thermoregulation conditions ( $G$ -test = 27.01,  $P < 0.001$ ) (Fig. 4). Similarly, the ability to thermoregulate offered no significant survival advantage for insects infected with *M. anisopliae* var. *acridum* (mean survival times of  $10.55 \pm 0.80$  and  $11.83 \pm 0.84$  days for constant and thermoregulation



**Fig. 4.** Cumulative percentage survival over 40 days of *Chorthippus parallelus* treated with blank oil, *Beauveria bassiana*, and *Metarhizium anisopliae* var. *acridum* when maintained under different thermal regimes: Constant, constant temperature (20 °C); Thermo, allowed to thermoregulate (20–45 °C). For all treatments,  $n = 60$ .

treatments, respectively; log rank statistic = 1.44;  $P = 0.23$ ). For *B. bassiana*, however, those insects able to thermoregulate survived significantly better than those maintained under constant temperature conditions (mean survival times of  $9.40 \pm 0.72$  and  $13.20 \pm 1.61$  days for constant and thermoregulation treatments, respectively; log rank statistic = 7.02;  $P = 0.008$ ) (Fig. 4). When insects were placed at a constant temperature of 25 °C after 40 days, all remaining insects in the pathogen treatments died within 5 days. Uninfected controls from both temperature regimes persisted beyond 10 days.

No sporulation of pathogens was observed on cadavers from either blank oil treatment. Percentage sporulation in *B. bassiana* treatments was 25.0% for the constant temperature regime and 16.7% for the thermoregulation regime ( $G$ -test = 4.011,  $P = 0.045$ ). For *M. anisopliae* var. *acidum*, figures were 13.0% and 15.0% for constant and thermoregulation regimes, respectively ( $G$ -test = 0.339,  $P = 0.56$ ). Sporulation was still observed in insects surviving beyond 40 days. Most insects not displaying signs of sporulation exhibited a black coloration indicative of secondary bacterial infection and decay (Elliot *et al.*, 2002). One *B. bassiana*-infected insect from day 2 produced spores of an as yet unidentified fungus. As in the previous study, a sarcophagid parasitoid emerged from one control grasshopper.

## Discussion

The field-derived body temperature model and the thermal gradient studies showed *C. parallelus* to be an active behavioural thermoregulator with a preferred body temperature in the range of 32–35 °C. This is lower than the 38–40 °C range found in some pest acridid species (Carruthers *et al.*, 1992; Inglis *et al.*, 1997; Blanford *et al.*, 1998; Blanford & Thomas, 1999b; Klass, 2004). However, Klass (2004) found similar values of 30–34 °C in non-pest acridids in Spain and previous studies of acridids in the U.K. indicate limited increases in body temperature above 35 °C for several species (Willott, 1997).

As indicated in the introduction, temperature and thermoregulation can have a marked impact on susceptibility of acridids (and other ectotherm hosts) to disease [see Thomas & Blanford (2003) for a review]. The exact mechanisms remain unclear in many cases but appear to depend on a combination of direct effects of temperature on the pathogen, with effects of temperature on host immune response (Ouedraogo *et al.*, 2003). In the current study, thermoregulatory behaviour enabled *C. parallelus* to maintain body temperatures around 32–35 °C. Infection with either *B. bassiana* or *M. anisopliae* var. *acidum* did not provoke a change in this normal thermal behaviour. This contrasts with several previous studies with other grasshopper or locust hosts that demonstrate behavioural fever responses to both these pathogens (Inglis *et al.*, 1996; Blanford *et al.*, 1998; Blanford & Thomas, 1999a, b; Elliot *et al.*, 2002; Wilson *et al.*, 2002; Bunday *et al.*, 2003).

Behavioural fever has been shown to provide important additional survival advantage beyond normal thermoregulation (Elliot *et al.*, 2002). Given that *C. parallelus* can thermoregulate and that both fungal pathogens studied cause substantial mortality (i.e. a fever response should potentially be possible and would appear desirable), the lack of fever response might suggest that the immune system of *C. parallelus* is failing to recognise the molecular 'signal' of infection due to evolutionary naivety to these particular pathogen strains. Alternatively, it is possible that the physiological risks involved in raising temperatures above the preferred range outweigh the advantages of such a febrile response; the increase in metabolic rate and other fitness costs associated with fever temperatures can be substantial in ectotherms (Muchlinski, 1985; Elia, 1992; Kluger *et al.*, 1998; Sherman & Stephens, 1998) including acridids (Elliot *et al.*, 2005).

Even in the absence of fever, normal set point body temperatures can still influence and potentially restrict pathogen growth (Blanford & Thomas, 1999b; Thomas & Blanford, 2003). In the current study it was observed that the opportunity to thermoregulate improved survival against *B. bassiana* but had no significant effect on *M. anisopliae* var. *acidum*. This suggests that the effect of thermoregulation is determined largely by the relative thermal sensitivities of the pathogens. That is, the optimum temperature for *B. bassiana* is approximately 25 °C, with an upper growth limit of  $\approx 36$  °C (Jaronski & Goettel, 1997). Thus, at the preferred body temperature of 32–35 °C, growth of *B. bassiana* within *C. parallelus* would be severely constrained affecting its virulence and, based on the sporulation data, reproductive fitness. For *M. anisopliae* var. *acidum*, on the other hand, the temperature optimum is 27 °C with an upper limit for growth approaching 40 °C (Thomas & Jenkins, 1997). Thus, a body temperature of 32–35 °C is unlikely to prevent growth to the same extent as it does for *B. bassiana*. Moreover, *in vitro* studies on the isolate used (IMI330189), show that growth rate at 20 °C is similar to that at 32–35 °C (Thomas & Jenkins, 1997). Hence, if the influence of temperature results largely from direct effects on fungal growth, rather than indirect effects mediated via host immune responses, it would be expected that patterns of mortality and sporulation in the constant and thermoregulation treatments would be more or less equivalent. This is consistent with the results of the final experiment. The differences observed between *Metarhizium*-treated insects kept under constant conditions or exposed to a gradient for a maximum of 2 h per day in the first experiment (differences that, comparing experiments, appear largely due to enhanced mortality in the gradient treatment), sit at odds with this but may be due to handling-induced stress increasing susceptibility.

Previous studies on other acridids have shown similar mortality patterns following infection with these fungal species. Sieglaff *et al.* (1997) found that *M. anisopliae* var. *acidum* was more virulent than *B. bassiana* to both the locust *Schistocerca americana* Drury and the North American temperate grasshopper *Melanoplus sanguinipes*

Fabricius, at a constant temperature of 30 °C. For *M. sanguinipes*, which has a higher preferred temperature range than *C. parallelus* (38–40 °C), Inglis *et al.* (1997) found that, while thermoregulation reduced the virulence of both pathogens, the impact was far greater on *B. bassiana*. Exposure to 35 °C for reduced periods affected mortality in *B. bassiana*-infected insects but not those treated with *M. anisopliae* var. *acridum*. When exposed to a range of oscillating temperature regimes, all with a mean of 25 °C, mortality of *M. sanguinipes* infected with *B. bassiana* was reduced when the maximum exceeded 30 °C (Inglis *et al.*, 1999). By contrast, mortality due to *M. anisopliae* var. *acridum* infection was only slightly reduced when the maximum temperature reached 40 °C.

Although a mixed-age population of only one grasshopper species was considered and the full range of factors that ultimately determine the impacts of pathogens on host population dynamics in the field were not explored, the current study has implications for a number of areas of general interest. For example, whilst 'climate matching' is generally considered important to predict those species most likely to establish in a new area, this study suggests that pathogen virulence and fitness might be greater if host and pathogen environments are mismatched. In this case, whilst a temperate pathogen, such as the isolate of *B. bassiana* used here, might be expected to be 'adapted' to the U.K. environment, its virulence and spore production were affected by normal thermal behaviour of the equally adapted *C. parallelus*. In contrast, the preferred body temperature of *C. parallelus* was not sufficiently high to constrain the development of the sub-Saharan, temperature-tolerant *M. anisopliae* var. *acridum*. This concept of mismatching is similar to the 'new associations' hypothesis in biological control, which argues that novel combinations of natural enemies and prey could prove more effective than the introduction of coevolved control agents from a pest species native range (Hokkanen & Pimental, 1984). Evidence in support of this from the biocontrol literature is somewhat equivocal (Waage & Greathead, 1988) and in a slightly different context, Ebert (1994) found that for parasites of *Daphnia magna* Straus (Cladocera: Daphniidae), locally adapted strains were more effective than novel strains. However, Milner *et al.* (1998) describe greater virulence for a 'new association' strain of *M. anisopliae* than for native isolates when tested on Australian termites.

The study also has implications for understanding impacts of climate change. The effects of general increases in ambient temperatures (Houghton *et al.*, 2001) and the frequency and duration of 'heatwaves' (Meehl & Tebaldi, 2004) under climate change scenarios, on both the migration and persistence of species and their associated pathogens, have yet to be determined. Current projections provide the potential for range increases in pest species (Sutherst *et al.*, 1995; Cannon, 1998; Baker *et al.*, 2000) and the emergence of new infectious diseases into wild populations (Daszak *et al.*, 2001). From this study, it is suggested that as ambient temperatures increase, temperate

pathogens might be suppressed or eliminated from communities as hosts achieve higher temperatures for a greater proportion of the day. In the longer term, this could lead to a shift in composition of microbial communities towards higher temperature tolerant pathogens that are currently marginal native isolates, or exotic Mediterranean/tropical strains. In the shorter term, whilst prevalence of lethal native pathogens and parasites appeared rather low in the grasshopper populations sampled here, the possibility remains that even subtle shifts in temperature or temperature extremes (Thomas & Blanford, 2003) could lead to release of host populations from pathogen control. Of course, the effect on particular species of any climatic change would be complex and dependent on a wide range of biotic and abiotic interactions (Stenseth *et al.*, 2002). While warmer temperatures may be advantageous to many species at the northern edge of their range, including those currently considered rare (Elmes & Free, 1994), fragmentation and degradation of habitat is likely to reduce the ability of less mobile species to expand their range, or escape associated changes in community composition (Hossell *et al.*, 2000). Nonetheless, it has been suggested that abiotic stress tends to have greater impact on higher rather than lower trophic levels (Menge & Sutherland, 1987). The influence of environmental change on predator/pathogen efficacy can have substantial indirect effects on communities through release or suppression of species at different trophic levels (Preisser & Strong, 2004). This suggests considerable value in further research on the role of environmental factors in host–pathogen interactions.

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### References

- Adamo, S.A. (1998) The specificity of behavioural fever in the cricket *Acheta domesticus*. *Journal of Parasitology*, **84**, 529–533.
- Baker, R.H.A., Sansford, C.E., Jarvis, C.H., Cannon, R.J.C. & MacLeod, A. & Walters, K.F.A. (2000) The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agriculture, Ecosystems, and Environment*, **82**, 57–71.
- Bateman, R.P., Carey, M., Moore, D. & Prior, C. (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology*, **122**, 145–152.
- Begon, M. (1983) Grasshopper populations and weather: the effect of insolation on *Chorthippus brunneus*. *Ecological Entomology*, **8**, 361–370.

- Blanford, S. & Thomas, M.B. (1999a) Role of thermal biology in disease dynamics. *Aspects of Applied Biology*, **53**, 73–82.
- Blanford, S. & Thomas, M.B. (1999b) Host thermal biology: the key to understanding host–pathogen interactions and microbial pest control? *Agricultural and Forest Entomology*, **1**, 195–202.
- Blanford, S. & Thomas, M.B. (2000) Thermal behaviour of two acridid species: effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. *Environmental Entomology*, **29**, 1060–1069.
- Blanford, S., Thomas, M.B. & Langewald, J. (1998) Behavioural fever in the Senegalese grasshopper, *Oedaleus senegalensis*, and its implications for biological control using pathogens. *Ecological Entomology*, **23**, 9–14.
- Bundey, S., Raymond, S., Dean, P., Roberts, S.K., Dillon, R.J. & Charnley, A.K. (2003) Eicosanoid involvement in the regulation of behavioural fever in the desert locust, *Schistocerca gregaria*. *Archives of Insect Biochemistry and Physiology*, **52**, 183–192.
- Cannon, R.J.C. (1998) The implications of predicted climate change for insect pests in the U.K., with emphasis on non-indigenous species. *Global Change Biology*, **4**, 785–796.
- Carruthers, R.I., Larkin, T.S., Firstencel, H. & Feng, Z. (1992) Influence of thermal ecology on the mycosis of a rangeland grasshopper. *Ecology*, **73**, 190–204.
- Chandler, D., Hay, D. & Reid, A.P. (1997) Sampling and occurrence of entomopathogenic fungi and nematodes in U.K. soils. *Applied Soil Ecology*, **5**, 133–141.
- Chapman, R.F. (1965) The behaviour of nymphs of *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) in a temperature gradient, with special reference to temperature preference. *Behaviour*, **24**, 283–317.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*, **78**, 103–116.
- Driver, F., Milner, R.J. & Trueman, W.H. (2000) A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. *Mycological Research*, **104**, 134–150.
- Ebert, D. (1994) Virulence and local adaptation of a horizontally transmitted parasite. *Science*, **265**, 1084–1086.
- Elia, M. (1992) Energy expenditure to metabolic rate. *Energy Metabolism: Tissue Determinants and Cellular Corollaries* (ed. by J. M. Kinney and H. N. Tucker), pp. 19–59. Raven Press, New York.
- Elliot, S.L., Blanford, S. & Thomas, M.B. (2002) Host–pathogen interactions in a varying environment: temperature, behavioural fever, and fitness. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **269**, 1599–1607.
- Elliot, S.L., Horton, C.M., Blanford, S. & Thomas, M.B. (2005) Impacts of fever on locust life history traits: costs or benefits? *Biology Letters*, **1**, 181–184.
- Elmes, G.W. & Free, A., eds (1994) *Climate Change and Rare Species in Britain*. HMSO, London.
- Fargues, J., Goettel, M.S., Smits, N., Ouedraogo, A. & Rougier, M. (1997) Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia*, **89**, 383–392.
- Hokkanen, H. & Pimental, D. (1984) New approach for selecting biological control agents. *Canadian Entomologist*, **116**, 1109–1121.
- Hossell, J.E., Briggs, B. & Hepburn, I.R. (2000) *Climate Change and UK Nature Conservation: a Review of the Impact of Climate Change on UK Species and Habitat Conservation Policy*. DETR, Bristol, U.K.
- Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van der Linden, P.J. & Dai, X. et al., eds. (2001) *Climate Change 2001: the Scientific Basis*. Cambridge University Press, Cambridge, U.K.
- Inglis, G.D., Duke, G.M., Kawchuk, L.M. & Goettel, M.S. (1999) Influence of oscillating temperatures on the competitive infection and colonisation of the migratory grasshopper by *Beauveria bassiana* and *Metarhizium flavoviride*. *Biological Control*, **14**, 111–120.
- Inglis, G.D., Johnson, D.L., Cheng, K.L. & Goettel, M.S. (1997) Use of pathogen combinations to overcome the constraints of temperature on entomopathogenic hyphomycetes against grasshoppers. *Biological Control*, **8**, 143–152.
- Inglis, G.D., Johnson, D.L. & Goettel, M.S. (1996) Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. *Biological Control*, **7**, 131–139.
- Jaronski, S.T. & Goettel, M.S. (1997) Development of *Beauveria bassiana* for control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada*, **171**, 225–237.
- Kemp, W.P. (1986) Thermoregulation in three rangeland grasshoppers. *Canadian Entomologist*, **118**, 335–343.
- Klass, J.I. (2004) *Biological control of locusts: using GIS to model spatiotemporal temperature-dependent variability in performance of a biopesticide*. PhD thesis, Imperial College, London.
- Kluger, M.J., Kozak, W., Conn, C.A., Leon, L.R. & Soszynski, D. (1998) Role of fever in disease. *Annals of the New York Academy of Sciences*, **856**, 224–233.
- May, M.L. (1985) Thermoregulation. *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Volume 4 Regulation: Digestion, Nutrition, Excretion* (ed. by G. A. Kerkut and L. I. Gilbert), pp. 507–522. Pergamon, Oxford.
- Meehl, G.A. & Tebaldi, C. (2004) More intense, more frequent, and longer lasting heat waves in the 21st Century. *Science*, **305**, 994–997.
- Menge, B.A. & Sutherland, J.P. (1987) Community regulation – variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist*, **130**, 730–757.
- Milner, R.J., Staples, J.A. & Lutton, G.G. (1998) The selection of an isolate of the hyphomycete fungus, *Metarhizium anisopliae*, for control of termites in Australia. *Biological Control*, **11**, 240–247.
- Muchlinski, A.E. (1985) The energetic cost of the fever response in three species of ectothermic vertebrates. *Comparative Biochemistry and Physiology. Part A*, **81**, 577–579.
- Ouedraogo, R.M., Cusson, M., Goettel, M.S. & Brodeur, J. (2003) Inhibition of fungal growth in thermoregulating locusts, *Locusta migratoria*, infected by the fungus *Metarhizium anisopliae* var. *acidum*. *Journal of Invertebrate Pathology*, **82**, 103–109.
- Ouedraogo, R.M., Kamp, A., Goettel, M.S., Brodeur, J. & Bidochka, M.J. (2002) Attenuation of fungal infection in thermoregulating locusts, *Locusta migratoria*, is accompanied by changes in hemolymphal proteins. *Journal of Invertebrate Pathology*, **81**, 19–24.
- Preisser, E.L. & Strong, D.R. (2004) Climate affects predator control of an herbivore outbreak. *American Naturalist*, **163**, 754–762.
- Samietz, J. & Köhler, G. (1998) The impact of behavioural thermoregulation on reproductive rates in a grasshopper. *Population and Community Ecology for Insect Management and Conservation* (ed. by J. Baumgartner, P. Brandmayr and F. Manly), pp. 63–73. Balkema, Rotterdam.
- Sherman, E. & Stephens, A. (1998) Fever and metabolic rate in the toad *Bufo marinus*. *Journal of Thermal Biology*, **23**, 49–52.
- Sieglauff, D.H., Pereira, R.M. & Capinera, J.L. (1997) Pathogenicity of *Beauveria bassiana* and *Metarhizium flavoviride*



- (Deuteromycotina) to *Schistocerca americana* (Orthoptera: Acrididae). *Journal of Economic Entomology*, **90**, 1539–1545.
- SPSS Inc. (2003) *SPSS 11.0 for Windows*. SPSS Inc., Chicago, Illinois.
- Stenseth, N.C., Mysterud, A., Ottersen, G., Hurrell, J.W., Chan, K.S. & Lima, M. (2002) Ecological effects of climate fluctuations. *Science*, **297**, 1292–1296.
- Sutherst, R.W., Maywald, G.F. & Skarrat, D.B. (1995) Predicting insect distributions in a changed climate. *Insects in a Changing Environment* (ed. by R. Harrington and N. E. Stirk), pp. 60–93. Academic Press, London.
- Thomas, M.B. & Blanford, S. (2003) Thermal biology in insect–parasite interactions. *Trends in Ecology and Evolution*, **18**, 344–350.
- Thomas, M.B. & Jenkins, N.E. (1997) Effects of temperature on growth of *Metarhizium flavoviride* and virulence to the variegated grasshopper, *Zonocerus variegatus*. *Mycological Research*, **101**, 1469–1474.
- Uvarov, B. (1966) *Grasshoppers and Locusts: a Handbook of General Acridology*. Cambridge University Press, Cambridge.
- Waage, J.K. & Greathead, D.J. (1988) Biological control – challenges and opportunities. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **318**, 111–126.
- Willott, S.L. (1997) Thermoregulation in four species of British grasshoppers (Orthoptera: Acrididae). *Functional Ecology*, **11**, 705–713.
- Willott, S.L. & Hassall, M. (1998) Life-history responses of British grasshoppers (Orthoptera: Acrididae) to temperature change. *Functional Ecology*, **12**, 232–241.
- Wilson, K., Thomas, M.B., Blanford, S., Doggett, M., Simpson, S.J. & Moore, S.L. (2002) Coping with crowds: density-dependent disease resistance in desert locusts. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 5471–5475.

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