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Host–pathogen interactions in a varying environment: temperature, behavioural fever and fitness

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We demonstrate how variable temperatures, mediated by host thermoregulation and behavioural fever, critically affect the interaction between a host (the desert locust, *Schistocerca gregaria*) and a pathogen (the fungus *Metarhizium anisopliae* var. *acridum*). By means of behavioural thermoregulation, infected locusts can raise their body temperatures to fever levels. The adaptive value of this behaviour was examined using three thermal regimes wherein maximum body temperatures achievable were: (i) below, or (ii) at normally preferred temperatures, or were (iii) unrestricted, allowing heightened fever temperatures. All infected locusts ultimately succumbed to disease, with median survival times of 8, 15 and 21 days post-infection, respectively. Crucially, only those locusts able to fever produced viable offspring. This represents, to our knowledge, the first demonstration of the adaptive value of behavioural fever following infection with a naturally occurring pathogen. By contrast, although normal host thermoregulation moderately reduced pathogen reproduction (by 35%), there was no additional negative effect of fever, resulting in an asymmetry in the fitness consequences of fever for the host and the pathogen. The dependency of the host–pathogen interaction upon external abiotic conditions has implications for how virulence and resistance are treated both theoretically and in the management of pests and diseases.

Keywords: environmental variability; virulence; resistance; thermoregulation; condition-dependency; locust biocontrol

1. INTRODUCTION

While there is a body of theory which considers adaptive changes in host resistance and pathogen or parasite virulence over evolutionary time-scales, the general assumption is that, over ecological time-scales, resistance and virulence are fixed at the onset of the interaction (Bull 1994; Ewald 1994; Frank 1996; Kraaijeveld *et al.* 1998; Fenner & Fantini 1999; Dieckmann *et al.* 2002). This assumption is challenged by empirical evidence that resistance or virulence may change during an ecological interaction due to intrinsic changes in the state of one of the organisms (Taylor & Read 1997; Pels & Sabelis 1999; Sokurenko *et al.* 1999; De Jong & Janss 2002). Meanwhile, extrinsic biotic and abiotic factors are generally viewed as 'setting the scene' for the interaction rather than having any explicit role once it is underway (Steinhaus 1960; Lewis & Tumlinson 1988; Karban & Myers 1989; Agrawal *et al.* 1999; Tollrian & Harvell 1999; Elliot *et al.* 2000). As a result, the effect of extrinsic factors on resistance or virulence during an interaction has received little attention. The possibility that natural enemies could increase their virulence in the presence of competing genotypes has only circumstantial backing (Elliot *et al.* 2002*b*) or evidence to the contrary (Read *et al.* 2002). There is better evidence of abiotic factors, particularly ambient temperature, affecting the progress and outcome of victim–enemy interactions (Fellowes *et al.* 1999; Stacey *et al.* 2002; and see below). Here, we consider a system in which a fluctuating thermal environment, mediated by host thermoregulatory behaviour (including behavioural fever), determines the course of a host–pathogen interaction.

In recent years, there has been considerable interest in biocontrol of locusts and grasshoppers (Orthoptera) using fungal pathogens (Lomer *et al.* 2001). The most significant advance has been the development of biopesticides containing the naturally occurring fungal pathogen of orthopterans, *Metarhizium anisopliae* var. *acridum* (*Metarhizium flavoviride* Gams and Rozsypal (Driver *et al.* 2000)) (Lomer *et al.* 2001). Whilst numerous laboratory and field trials have demonstrated efficacy of these biopesticides in locust and grasshopper biocontrol, the speed of kill following application is highly variable (Hunter *et al.* 1999; Langewald *et al.* 1999; Lomer *et al.* 1999, 2001). This has been found to be due not to poor quality product or application, but to variable ambient temperatures and host thermoregulatory behaviour (Blanford *et al.* 1998, 2000; Blanford & Thomas 1999*a*, 2000; Scanlan *et al.* 2001).

Three processes contribute to the influence of ambient temperature in interactions between Orthoptera and fungal pathogen interactions (Carruthers *et al.* 1992; Inglis *et al.* 1996; Blanford & Thomas 1999*a*, 2001). First, temperature has a direct effect on the ability of the pathogen to infect and grow within the host (Thomas & Jenkins 1997; figure 1). Thus, *M. anisopliae* var. *acridum* grows best (and is most virulent) around 27–30 °C. However, most orthopterans (especially those targeted for biocontrol) are active behavioural thermoregulators and, like many other ectotherms, select a thermal environment

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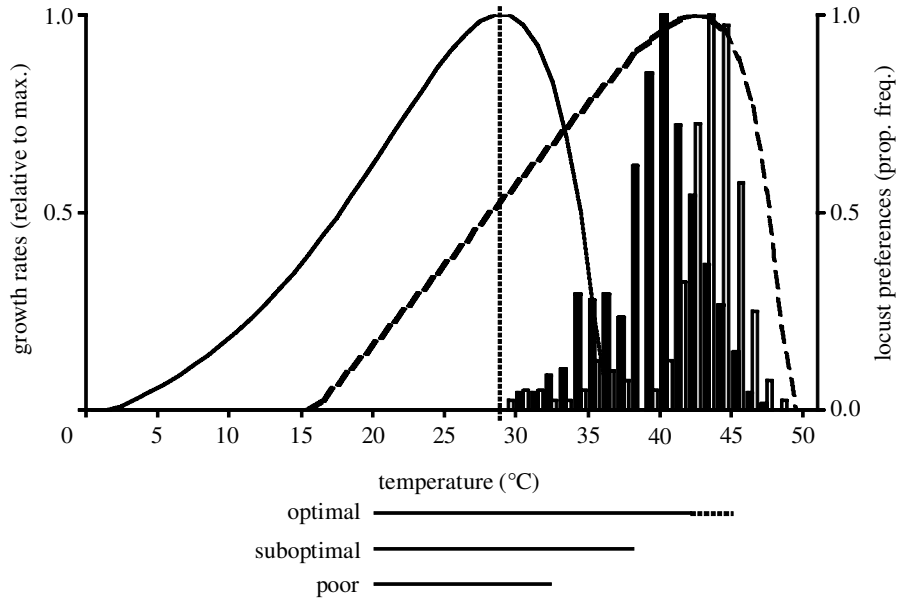


Figure 1. Comparison of thermal growth profile of *Metarhizium anisopliae* var. *acridum*, thermal development rate profile of nymphal *Schistocerca gregaria* from hatchling to adult and thermal preferences of healthy and *Metarhizium*-infected *S. gregaria*. (Black bars, uninfected locusts; white bars, infected locusts; solid line, pathogen growth; dashed line, locust development.) Below the graph are shown the intended body temperatures available in the 'poor', 'suboptimal' and 'optimal' treatments in the experiment.

close to a desired body temperature and then make subtle adjustments in posture to balance heat loss and gain. Given suitable environmental conditions, this regulatory behaviour allows orthopterans to maintain their body temperature close to 38–40 °C for large parts of the day (Carruthers *et al.* 1992; Lactin & Johnson 1996, 1998; Blanford & Thomas 1999*a*,*b*). As the upper threshold for *M. anisopliae* var. *acridum* growth is *ca.* 37 °C (Thomas & Jenkins 1997; figure 1), maintenance of such body temperatures through thermoregulation restricts pathogen growth inside the host, leading to substantial delays in fungus-induced mortality (Inglis *et al.* 1996, 1997*a*; Blanford & Thomas 1999*b*, 2000). This effect is compounded by the third factor, host behavioural fever, whereby orthopterans can elevate their body temperatures to 42–44 °C in response to disease challenge (Inglis *et al.* 1996; Blanford *et al.* 1998; Blanford & Thomas 1999*a*, 2000; figure 1). These fever temperatures are further above the pathogen's upper growth threshold and may increase the functioning of the host's immune system (R. M. Ouedraogo, personal communication). For *Metarhizium*, however, these temperatures are not lethal and there is no evidence of orthopterans curing themselves through fever (although high body temperatures can eliminate other fungal pathogens (Carruthers *et al.* 1992)). Thus, the pathogen still has the potential to kill the host if ambient temperatures return to permissive levels. The overall speed of kill (and indeed whether the pathogen ultimately kills the host at all) is, therefore, critically determined by daily temperature fluctuations: for example, the degree to which daytime periods of thermoregulation, with nil pathogen growth or even decay, balance growth at night when hosts cannot thermoregulate (Blanford & Thomas 1999*a*,*b*, 2000). What remains unclear, however, is the extent to which fever itself provides additional survival advantages to the host above and beyond normal thermoregulatory behaviour; the normally preferred body temperatures are already at or above the upper limit for pathogen growth, so what is the benefit of a (potentially costly) further increase in temperature through a fever response?

Many, but not all, invertebrate and vertebrate ectotherms are capable of behavioural fever (Kluger *et al.* 1975; Covert & Reynolds 1977; Watson *et al.* 1993) and ectotherms have been used as models to explore the adaptive value of (physiological) fever to endotherms (Kluger 1978; Banet 1986; Blatteis 1986). While this approach has been criticized as extrapolative (Blatteis 1986), it can still provide insights into a parallel phenomenon which employs some similar physiological pathways (Kozak *et al.* 2000). Experiments designed to examine the effects of fever have tended either to limit fever using antipyretic drugs (generally for endotherms) or to restrict fever by fixing available ambient temperatures at set-points (for ectotherms). The results for endotherms have been inconclusive (Blatteis 1986). For ectotherms, while some studies indicate fitness benefits, to our knowledge no study has clearly demonstrated the adaptive value of fever in terms of fitness correlates such as survival and reproduction using natural routes of infection (as opposed, for example, to invasive injection), allowing animals to regulate their body temperatures themselves, and using a system where fever has been shown as a natural response in the field (Kluger *et al.* 1975; Covert & Reynolds 1977; Louis *et al.* 1986; Boorstein & Ewald 1987). Critically, while the ability to thermoregulate has been shown to have fitness benefits for infected animals (e.g. Blanford & Thomas 2001), to our knowledge no study to date has attempted to partition the effect of behavioural fever from normal thermoregulatory behaviour.

In this study, we examine whether fever (in this instance a behavioural trait) is adaptive to the host and what the consequences are to the pathogen. To investigate this we used the desert locust, *Schistocerca gregaria* (Forskål), and

the fungal pathogen, *M. anisopliae* var. *acridum*. We allowed the pathogen to infect through its natural process of germination and penetration of the cuticle and permitted the locusts to thermoregulate freely, but limited the temperature maxima they could reach, in order to partition the effects of normal preferred body temperatures from enhanced fever temperatures. We then assessed survival and reproduction as estimates of host fitness. We hypothesized that the fluctuating thermal environment (mediated by thermoregulatory behaviour) would determine the progress and outcome of the interaction, that fever would be adaptive to infected hosts, and (parsimoniously) that fever would have negative fitness consequences for the pathogen. We interpret our results in terms of the probable pattern of selection on pathogen virulence and host resistance (fever).

2. MATERIAL AND METHODS

(a) *Experimental design*

The study comprised four replicate blocks of six treatments. Each replicate consisted of a cage with 10 male and 10 female 5th instar *S. gregaria*, acquired as 4th instars (Blades Biological, Edenbridge, Kent, UK) and inoculated 2 to 5 days after moulting to 5th instar. Blocks were staggered to start on different dates over a 4 day period, ensuring a similar physiological age for each animal and allowing at least 10 days for infections to establish before final moult to adults. Locusts in infected treatments were inoculated with 2×10^4 conidia of *M. anisopliae* var. *acridum* (IMI 330189, the strain used in one of the locust biopesticide products) in 2 μl of peanut oil applied to the base of the dorsal pronotal shield with a micropipette (Prior *et al.* 1995). This process of inoculation allows for the dose to be controlled but still requires that the fungus invade the host through natural mechanisms of infection (i.e. germination of conidia, production of appressoria, growth of penetration peg, action of cuticle degrading enzymes, etc. (Clarkson & Charnley 1996)). Controls were similarly treated with blank peanut oil. Locusts were then placed in aluminium cages with perforated floors and glass fronts. These were held in a climate room set at 20 °C (±1 °C), a temperature at which pathogen growth is intermediate (Thomas & Jenkins 1997; figure 1).

Each cage was fitted with a light bulb three-quarters of the way up the back wall. Different bulb wattages were used to generate three daytime thermal regimes: 40 W for 'optimal', 25 W for 'suboptimal' and 12 W for 'poor'. These treatment names were ascribed to relate to the body temperatures which an infected locust could achieve, the optimal treatment allowing fever temperatures, suboptimal allowing normally preferred (but not fever) temperatures, and poor limiting locusts below their normally preferred range (figure 1). Body temperature maxima were limited by restricting the degree to which locusts could bask near the bulb, using galvanized steel mesh (6 mm square grid) placed around each bulb and taped to the cage wall as a shield to keep locusts at least 2 cm away from the bulb. Plastic mesh sheets were placed in each cage as a climbing frame, from floor to ceiling and cut out around the bulb shield. A thermal gradient was thus created, allowing locusts to select temperatures within the restrictions set by the treatments. Under these conditions, *Metarhizium*-infected *S. gregaria* will attempt to thermoregulate to fever temperatures (e.g. Blanford & Thomas 1999*a*). Cage bulbs were set on timers to allow 9 h daytime thermoregulation with the remaining time at the background room temperature of 20 °C (± 1 °C). Dawn and dusk lighting were simulated for 1 h 30 min before and after 'daytime', using two sets of three 60 W bulbs in room corners. To check the body temperatures achievable, live locusts were secured with cotton thread in various positions in the cages (see below) and left for *ca.* 30 min for body temperatures to stabilize. Temperatures were recorded using a copper-constantan thermocouple (diameter 0.125 mm) linked to a digital thermometer, the thermocouple tip inserted in the thorax to a depth of 2 mm (Blanford & Thomas 1999*a*). The positions and the recorded temperatures are shown in figure 2.

The locusts were fed *ad libitum* on a diet of *ca.* 12 day old wheat seedlings, replaced daily, and wheat bran. Mortality and moulting were scored daily, including whether death was before, during or after moult. Adults were assessed for whether any defects had been acquired during moult. To assess the presence of haemocyte nodules (aggregations of haemocytes around foreign particles such as *Metarhizium* hyphal bodies) in shed cuticles, half the thoracic section of each was mounted on a slide in lactophenol cotton blue for microscopic examination. Dead locusts were placed on filter paper in aerated Petri dishes for 2 days at 20 °C to allow development of the red coloration characteristic of *Metarhizium* colonization of cadavers. The filter paper was then moistened with sterile distilled water to see if *Metarhizium* sporulated from the cadavers or if they were colonized by bacteria. The experiment ran for 53 days post-inoculation, whereupon cage bulbs were switched off to leave a constant temperature of 20 °C: locusts which subsequently died and sporulated were taken to have remained infected until the end of the experiment.

Once mating had been observed in the cages, trays of moist sterile sand were placed in the two optimal treatments (very few treated animals were left in the other thermal regimes) and left for *ca.* 4 days to allow oviposition. For logistical reasons it was not possible to quantify production of viable offspring but whether any hatchlings emerged was noted.

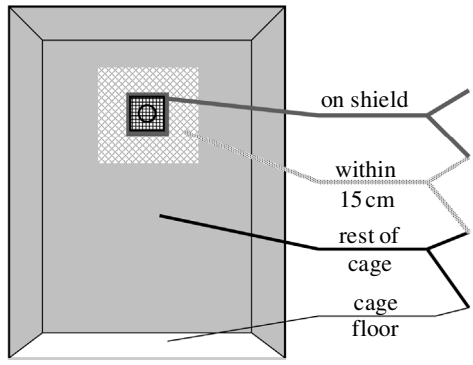
(b) *Observations of thermoregulatory behaviour*

On days 5 and 6 post-inoculation (having allowed time for the infection to establish), hourly observations of the positions of each locust were made during the day, beginning 30 min before cage bulbs came on in the morning. Locusts were recorded as being in one of four zones: on the bulb shield, within 15 cm of the shield, on the plastic mesh or cage walls/roof, or on the cage floor (usually feeding) (see figure 2). The body temperatures (see above) were taken from the borders of these zones, including the hottest and coolest parts of the cage (on top of the bulb shield and on the cage floor near the front).

3. RESULTS

(a) *Behavioural observations*

The positions of locusts within the cages on days 5 and 6 post-inoculation are summarized in figure 3. Pairwise comparisons were made with two-tailed sign tests, equal values removed to give n comparisons (Sokal & Rohlf 1995, p. 444). Observations made before the bulbs came on were excluded from analyses. In each thermal regime, infected locusts spent less time feeding than their uninfected counterparts ($p < 0.01$, $n_{\text{optimal}} = 61$, $n_{\text{suboptimal}} = 61$, $n_{\text{poor}} = 63$), consistent with previous observations (Moore *et al.* 1992; Seyoum *et al.* 1994). In comparisons of the three control treatments, locusts spent less time raising



	poor	suboptimal	optimal
on shield	30.6–33.8	39.7–44.3	44.2–63.0
within 15 cm	23.9–26.3	26.9–31.0	30.1–32.6
rest of cage	25.0–27.1	27.7–29.8	30.1–37.5
cage floor	21.0–24.0	22.0–23.8	23.5–24.8

Figure 2. Thermal map of locust cages used in experiment, by treatment and by position in cage. Values given are the ranges of body temperatures (i.e. maximum and minimum over eight cages given a particular thermal regime) for each zone in which behavioural observations were made (figure 3).

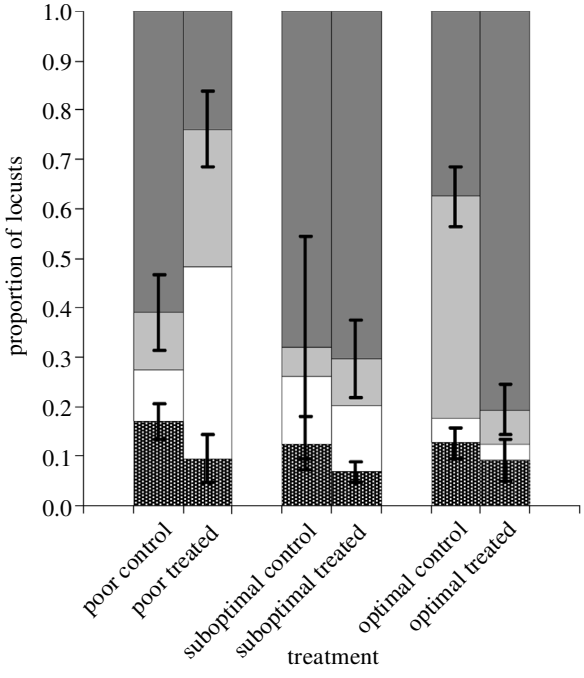


Figure 3. Thermoregulatory behaviour of infected and uninfected *Schistocerca gregaria* in cages. Shown are hourly observations, pooled by treatment, of locust positions relative to heat sources (light bulb covered with a mesh shield), on days 5 and 6 post-inoculation (dark grey, on shield; light grey, within 15 cm of shield; white, on mesh/sides; hatched, feeding). Standard error bars are shown for proportions of locusts on the shield or feeding.

their body temperatures on the hottest bulbs (optimal < suboptimal, $p < 0.01$, $n = 72$; optimal < poor, $p < 0.01$, $n = 69$). These results confirm that the availability of preferred ambient temperatures during the day was unrestricted in the optimal regime, while healthy insects were striving to raise or keep their body temperatures at 38–39 °C in the suboptimal and poor treatments. In the optimal treatment, infected locusts spent more time on the shields than did the controls ($p < 0.01$, $n = 71$), implying that they spent more time basking so as to achieve fever temperatures. In the suboptimal treatment there was no significant difference between infected and uninfected locusts ($p > 0.05$, $n = 72$), implying that healthy insects could only just reach their preferred body temperatures (figures 1 and 2) and infected insects had to accept the same (suboptimal) temperatures. In the poor treatment, infected locusts spent less time close to the heat source than did the controls ($p < 0.01$, $n = 71$). The thermal regimes were, therefore, as intended. The behaviour of infected locusts in the poor treatment is discussed below.

(b) *Locust survival*

Only four of the 234 uninfected control locusts died within the 53 days for which the experiment ran (figure 4), giving median or mean survival times of over 53 days for pooled replicates (Kaplan–Meier survival analyses, SPSS for Windows v. 6.1). By contrast, the only infected locusts to survive to the end of the experiment were seven out of the 81 animals in the optimal treatment. Estimated median survival times were 8 days (95% CI of 8 days) in the poor treatment, 15 days (95% CI of 13–17 days) in the suboptimal treatment and 21 days (95% CI of 20–22 days) in the optimal treatment (all significantly different from one another and from corresponding controls, by pairwise log-rank comparisons $p < 0.000\,05$). Variation in survival time (95% CI) of infected insects was greatest in the suboptimal thermal regime, indicating that variation between cages in available body temperature maxima was most critical in the range spanning normal and fever temperatures. (For the suboptimal treatments, the maximum body temperature of 44.3 °C given in figure 2 was a control cage: maxima for cages with infected locusts were 39.5, 41.5, 42.9 and 43.0 °C, i.e. generally at or below fever temperatures of 42–44 °C. Critically, these measurements were made on the hottest part of the shields in a very limited area directly above the bulbs, so the maxima would only have been achievable by a few locusts at a time, compared with the optimal treatment where fever temperatures were available to all locusts throughout the day.) The seven infected animals which survived to the end of the experiment died within three days once the cage bulbs were switched off, their cadavers sporulating once in humid conditions. They had, therefore, not rid themselves of the infection despite being able to fever for 53 days. That said, haemocyte nodules were found on all of the shed cuticles of infected locusts which moulted, and on none of the uninfected locusts. These structures represent the encapsulation of foreign particles as a component of the host's immune response, implying that some

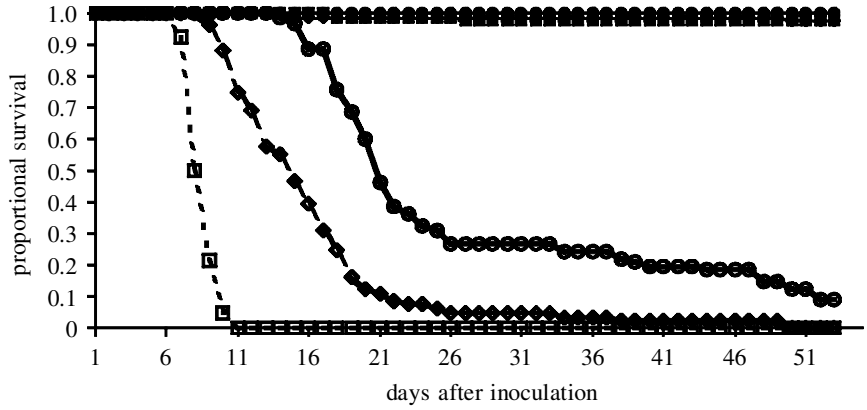


Figure 4. Effect of thermal environment on survival of infected and uninfected locusts. Shown is the proportional survival of 5th instar *Schistocerca gregaria* which were either inoculated with *Metarhizium anisopliae* var. *acridum* or were uninfected, and then were held in locust cages with heat sources which provided a thermal environment either poor, suboptimal or optimal for thermoregulation of infected locusts to fever temperatures (open squares, poor infected; open diamonds, suboptimal infected; open circles, optimal infected; filled squares, poor control; filled diamonds, suboptimal control; filled circles, optimal control).

Table 1. The effect of thermoregulation and fever on success of final instar moult and related mortality of infected and uninfected locusts. Shown are percentages with numbers of individuals in parentheses.

	death								
treatment	before moult		during moult		after moult		survival		*n*
poor treated	100	(80)	0	(0)	0	(0)	0	(0)	80
suboptimal treated	49	(40)	20	(16)	32	(26)	0	(0)	82
optimal treated	0	(0)	6	(5)	85	(69)	9	(7)	81
controls (pooled)	0	(0)	0.5	(1)	1	(2)	98.5	(219)	222

shedding of pathogen may have occurred at moult, but not sufficient to cure the host.

(c) *Locust moulting*

The median onset of moult was delayed 2 to 3 days in infected insects compared with controls (excluding deaths prior to moult), in both suboptimal and optimal regimes (Kaplan–Meier survival analysis in SPSS, with log-rank comparisons at $p < 0.000\,05$). Of the infected locusts, all those in the poor treatment died before moulting to adults (table 1). Of those in the suboptimal treatment, 48% died prior to moulting, 20% during moulting and 32% subsequently as adults. For the locusts in the optimal treatment, none died before moulting, 6% died during moulting, while 85% died as adults and 9% survived to the end of the experiment. A 3×4 test of independence (Sokal & Rohlf 1995, p. 737), showed these frequencies to be associated with thermal regime ($p \ll 0.001$ as $G = 246.6$ is greater than $\chi^2_{0.001[6]} = 22.5$). Locusts which died during moulting ranged from animals which had only begun to shed the cuticle from the abdomen to animals which had moulted but remained with the cuticle attached, usually to their wings, debilitating them. Every infected locust which managed to moult had distorted wings and sometimes legs. This ranged from heavily stunted and crinkled wings to cases where the wings were not folded correctly, so collecting excretia in the tips. In the suboptimal treatment, death usually followed within three days of moulting, while in the optimal treatment death was on average 8 days later, although some individuals survived much longer.

(d) *Locust reproduction*

The infected insects which survived into adulthood subsequently matured, mated and oviposited *ca*. 30 days post-inoculation, producing substantial numbers of offspring. These numbers were not assessed for logistical reasons but there was no difference from controls apparent (subsequent repetition of the two optimal treatments with hatchling counts supports this (Elliot *et al.* 2002*a*)).

(e) *Pathogen sporulation*

Almost all (99%) of the infected locusts which died prior to moulting turned red, indicative of complete colonization of the cadaver by *Metarhizium*, and sporulation was observed over the whole body after subjection to humid conditions (figure 5*a*). Most (67%) animals which died during moulting had developed a black coloration prior to death, indicative of secondary bacterial infection, and did not sporulate in humid conditions but simply putrified. An additional 24% also went black and putrified but sporulated partially, this being restricted to the extremities of the locusts (the antennae, legs and wing buds). The remaining 9% sporulated completely. Of the adults which died, 13% did not sporulate at all, 25% sporulated partially and 62% sporulated completely. Of these, only those which died shortly after moulting putrified, while those that died later were more likely to sporulate partially or completely. Treating these same data with respect to the thermal regimes (figure 5*b*), *Metarhizium* sporulation from cadavers was 100% complete in the poor regime, and 64–65% complete and 18–21% partial when locusts were given suboptimal or optimal thermal conditions. These

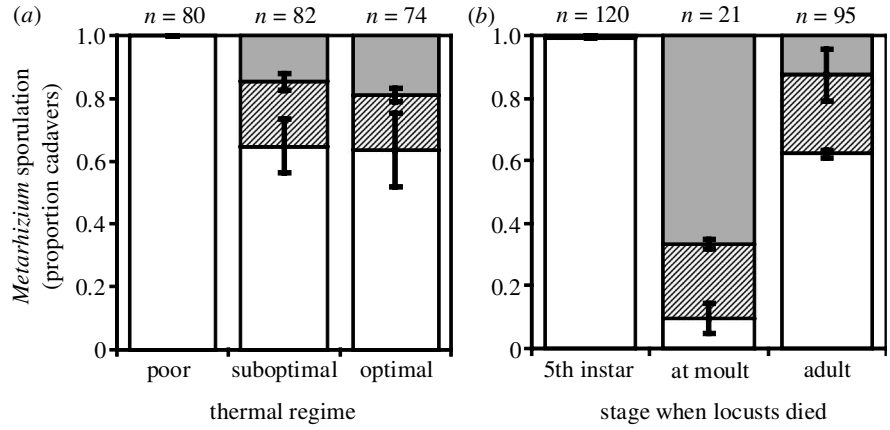


Figure 5. (*a*) Effect of host thermoregulation on pathogen fitness, as estimated by success or otherwise of sporulation of *Metarhizium anisopliae* var. *acridum* from cadavers of *Schistocerca gregaria* from a laboratory experiment in which the ability of infected locusts to thermoregulate was poor, suboptimal or optimal. (*b*) The same data according to when locusts died relative to moult. (Grey bars, no sporulation; diagonal hatched bars, partial; white bars, complete.)

data were subjected to 3×3 tests of independence (Sokal & Rohlf 1995, p. 737) which demonstrated that frequency of sporulation was not independent of treatment ($p \ll 0.001$ as $G = 55.6$ is greater than $\chi^2_{0.001[4]} = 18.5$) or stage at death ($p \ll 0.001$ as $G = 117.4$ is greater than $\chi^2_{0.001[4]} = 18.5$). Pairwise (i.e. 2×3) tests of independence were significant at $p < 0.001$ ($G > 29.5$ so greater than $\chi^2_{0.001[1]} = 10.8$) for all such comparisons except for sporulation frequencies in suboptimal versus optimal treatments ($G = 0.639$). The Williams correction was unnecessary as it did not qualitatively change the results of the analyses.

4. DISCUSSION

This study was intended to explore the critical role of ambient temperature in a host–pathogen interaction as mediated by host thermoregulation and behavioural fever, and to test the adaptive value of fever to the host and the fitness consequences to the pathogen. Experimental conditions were set such that for 15 h during the night, ambient temperatures permitted pathogen growth within the locust host (see Thomas & Jenkins 1997; figure 1). For 9 h during the day, the locusts could thermoregulate but only to imposed maxima (confirmed by measurements of body temperatures and behavioural observations of locusts). This set-up allows discrimination between effects of normal thermoregulatory temperatures which are already very high for the pathogen, and increased behavioural fever temperatures on the host–pathogen interaction.

(a) *Locust behaviour*

While the behavioural observations of locust thermoregulation were primarily intended to confirm that the thermal regimes were as planned, the observation that infected locusts in the poor treatment spent less time near to the bulb than did the controls is curious. One explanation is that under this thermal regime which clearly favoured pathogen growth, locusts were too sick 5 and 6 days post-inoculation to thermoregulate effectively. An intriguing alternative possibility is that this represents afebrile behaviour, with the hosts attempting to limit pathogen growth by thermoregulating to body temperatures below the pathogen's optimum of 28 °C. An afebrile response has been demonstrated in bumble-bees infected with parasitoids (Müller & Schmid-Hempel 1993) but not, to our knowledge, in orthopterans. This is the subject of future study.

(b) *Adaptive value of behavioural fever*

For the infected locusts in this experiment, the ability to thermoregulate was crucial for any chance of survival. Without this, as in the poor treatment, death due to mycosis was rapid. Allowed to reach body temperatures which are preferred by healthy hosts but not allowed to fever freely (the suboptimal treatment), locusts survived for longer but still died before reproduction. The large variation in survival times for the suboptimal treatment highlights the sensitivity of the host–pathogen interaction to slight variations in temperature around the interface between normal and fever temperatures. Critically, when behavioural fever was unrestricted, some locusts were able to moult, mature and reproduce. Previous studies have either demonstrated benefits to the host of active thermoregulation but without discriminating between normal thermoregulatory behaviour and fever (e.g. Inglis *et al.* 1997*b*; Blanford & Thomas 2001) or have used set-point thermal regimes which mimic elevated body temperatures (e.g. Inglis *et al.* 1996). We therefore believe this study to be the first demonstration of the effects of behavioural fever *per se*, on host fitness. How this result translates exactly to fitness under the range of possible conditions that might be experienced in the field is unclear. Factors such as pathogen dose, timing of infection, day length (influencing duration and extent of the fever response) and night-time temperatures (particularly whether they allow for significant periods of pathogen growth or not), will all combine to determine the ultimate course of an infection. Notwithstanding this, in our experimental system, fever was necessary to achieve some measurable fitness. As *Metarhizium* is not transmitted vertically, this fitness benefit is not compromised by transfer of infection to the offspring.

Injection of *S. gregaria* with the fungal wall protein laminarin has been shown to stimulate individuals to

select fever temperatures (K. Charnley, personal communication), implying that behavioural fever is under the control of the host. This supports the hypothesis that behavioural fever is a (host-mediated) adaptive response to infection. Interestingly, however, despite being able to fever, no locust was able to cure itself of the infection. The presence of haemocyte nodules (perhaps containing fungus particles) on the interior of ecdysed cuticles indicates that locusts may be able to shed pathogen at moult, but whether they can eliminate the pathogen altogether through successive moults if infection occurs at an earlier developmental stage is unclear. This, together with the observed delay in moulting in infected locusts are subjects of ongoing investigation.

(c) *Fitness consequences of fever to the pathogen*

If fever is adaptive for an infected host, then the first expectation must be that it negatively affects the pathogen's fitness. In the regimes where the host could thermoregulate very little (poor) or only to normally preferred body temperatures (suboptimal), comparison of sporulation of *Metarhizium* from infected cadavers does suggest that host thermoregulation has negative consequences for the pathogen; thermoregulating insects showed a significant reduction in percentage of sporulation with many cadavers lost to competing bacteria. Allowing locusts to elevate their body temperatures to the higher fever temperatures (optimal) had no additional effect on pathogen sporulation, however. Bacterial infection was an uncontrolled factor in this experiment but the phenomenon has been observed in other Orthoptera during field trials with the locust biopesticide, particularly during moulting (S. Blanford and S. L. Elliot, personal observation). Even with these secondary infections, the effect of thermoregulation and fever on pathogen fitness is much less than the effect on host fitness (for whom no thermoregulation is catastrophic). Therefore, while there may be selection on the pathogen to prevent thermoregulation and kill the host rapidly, this selection is expected to be considerably weaker than the pressure on the host to fever. However, for pathogens less able to resist elevated fever temperatures (e.g. the fungus *Beauveria bassiana*), the hypothesis probably does hold (Inglis *et al.* 1996).

(d) *Conclusions*

We have demonstrated that ambient temperatures, mediated by host thermoregulatory behaviour, can be critical in determining the progress and outcome of a host–pathogen interaction and that host behavioural fever is adaptive. We are currently investigating the costs of this defence mechanism. In addition, we expect selection on the pathogen to counteract behavioural fever to be weak.

The body of theory on the evolution of resistance and virulence has implicitly assumed these parameters to be fixed over the lifetime of a victim–enemy interaction. The results of the current study (and others such as Tanada & Chang 1968; Carruthers *et al.* 1985, 1992; Inglis *et al.* 1997*b*; Karban 1998; Blanford & Thomas 1999*a*; Fellowes *et al.* 1999), are clearly a challenge to this assumption and make a strong case for the incorporation of variable environmental conditions, particularly temperature, in theoretical and empirical work on victim–enemy interactions.

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REPORT

Fever and phenotype: transgenerational effect of disease on desert locust phase state

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Abstract

Natural enemy attack can cause transgenerational shifts in phenotype such that offspring are less vulnerable to future attack. Desert locusts (*Schistocerca gregaria*) show density-dependent variation in their resistance to pathogens, such that they are less vulnerable to pathogens when in the high-density gregarious phase state (when they would probably be more exposed to pathogens) than when in the solitarious phase state. We therefore hypothesized that infected gregarious parents would maintain this phenotype in their offspring. We infected gregarious desert locust nymphs with the fungal pathogen *Metarhizium anisopliae* var. *acridum*, and allowed them to survive to reproduction by means of behavioural fever. The phase state of the locust offspring was assessed by their colouration and behavioural assays. Contrary to our hypothesis, we found an increase in solitarization in the infected population (14.6% solitarious offspring from infected parents, vs. <2% from uninfected counterparts at equivalent density). In a second experiment, we simulated behavioural fever temperatures and obtained a similar result (13.6% solitarious offspring vs. 4.4% from controls), implying that the phenomenon is probably a side-effect of the hosts' fever response. Identification of this novel environmental factor affecting locust phase state could have important implications for the biological control of these major pests.

Keywords

Behavioural fever, biological control, entomopathogenic fungi, locusts, maternal effects, phase polyphenism, phenotypic plasticity.

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INTRODUCTION

A range of insects show density-dependent phase polyphenism whereby group-living '*gregaria*' have different morphologies, colouration and behaviour from low density '*solitaria*', with intermediate phases filling the continuum (Applebaum & Heifetz 1999). The desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae), is the classic example of such an organism. A locust's phase at eclosion will have been determined by its parents' phase state and their experience of crowding with other locusts prior to oviposition (Islam *et al.* 1994a,b; Bouaïchi *et al.* 1995). In the subsequent development of nymphs, a greater or lesser degree of exposure to other locusts (in particular associated tactile stimuli) will affect the locust's phase (Roessingh *et al.* 1998; Simpson *et al.* 2001). Phase state is a combination of characters, the most labile of these being the locust's behaviour: a cryptic green *solitaria* hatchling will behave just like a *gregaria* after a few hours of contact with other locusts (Bouaïchi *et al.* 1995), but will only begin to acquire the *gregaria* morphology and colouration at subsequent moults. Furthermore, *solitaria* desert locusts have been shown to be more susceptible to a mitosporic fungal pathogen, *Metarhizium anisopliae* var. *acridum*, than *gregaria* individuals and to invest less in haemolymph antimicrobial activity (Wilson *et al.* 2002). This is consistent with the density-dependent prophylaxis hypothesis that animals living at higher densities are at greater risk of infection so should invest more in resistance (Wilson & Reeson 1998), and has implications for the use of *M. anisopliae* var. *acridum* as the major agent in locust and grasshopper biocontrol (Lomer *et al.* 2001).

More generally, an organism's phenotype can have a profound effect upon its vulnerability to natural enemies. In response to non-lethal attack by a predator or parasite, an organism may induce defences which reduce its future vulnerability (e.g. Tollrian & Harvell 1999) or may induce

such defences in its offspring (e.g. Roberts 1983; Agrawal *et al.* 1999; Moret & Schmid-Hempel 2001). There is accumulating evidence that such maternal inheritance may be adaptive (Agrawal *et al.* 1999; Agrawal 2001). Linking these two concepts, given that *solitaria* locusts are more susceptible to pathogens such as *Metarhizium*, we might expect an increase in gregarization of locust offspring produced by an infected *solitaria* adult. At the very least, and considering that there are many interacting cues determining phase state, we would expect *Metarhizium*-infected gregarious adults to maintain this phase in their offspring to conserve the enhanced resistance.

Here we present the results of a study that, contrary to this hypothesis, indicates increased solitarization of locust offspring from infected gregarious adults. In addition, we identify that this response appears to be a consequence of behavioural fever, one of the key defence mechanisms which locusts and grasshoppers employ in resisting pathogens (Inglis *et al.* 1996; Blanford & Thomas 1999a,b; Elliot *et al.* 2002).

METHODS

We conducted two experiments which, together, allowed us to investigate the effects of infection on the phase state of locust offspring (a correlate of susceptibility to pathogens) and to determine the mechanisms involved. In the first experiment, we inoculated gregarious locusts with *Metarhizium*, allowed them to survive to reproduction by permitting behavioural fever (Elliot *et al.* 2002) and assessed the phase state of their offspring. In addition, we tested for the effects of disease-induced reduction in adult locust density during sexual maturation and reproduction. That is, though fever prolongs survival and allows some infected locusts to reproduce successfully, they still succumb to the disease and suffer enhanced mortality (Elliot *et al.* 2002). Given the potential importance of density in phase change, we needed to correct for such influences to separate any direct effects of infection on phase state, from indirect effects via changes in density.

In the second experiment, we imposed an artificial, but realistic, simulated fever regime on uninfected locusts and again assessed the phase state of their offspring. This enabled us to test the effects of behavioural fever temperatures, independent of disease itself.

Experiment 1

The protocol followed that of Elliot *et al.* (2002). As such, *gregaria* 4th instar *S. gregaria* were acquired from Blades Biological (Edenbridge, Kent, UK), and held in standard aluminium locust cages with mesh climbing frames and light bulbs. The experiment began shortly after locusts had moulted to 5th instar. We established four treatments, each consisting of a population of locusts at an equal sex ratio which were provided daily with fresh wheat seedlings and bran as food. Locust cages were assigned to four replicate blocks and held in a climate room with a background temperature of 20 ± 1°C. Each cage was equipped with a 40 W light bulb two-thirds of the way up the cage back, to allow thermoregulation. These bulbs were switched on for 9 h per day. Each bulb was covered by a steel mesh shield restricting locusts to a distance of 2 cm from the light bulb, thus allowing thermoregulation to typical fever temperatures for up to 9 h per day but avoiding competition for hot local environments (see Elliot *et al.* 2002). To initiate infection, insects were treated with 2×10^4 conidia of *M. anisopliae* var. *acridum* (IMI 330189) (from the same batch and with >90% viability) in 2 μl of peanut oil applied under the dorsal pronotal shield with a micropipette (Prior *et al.* 1995). Controls received 2 μl of oil with no inoculum.

The principal treatment ('infected') consisted of populations of 20 locusts per cage, inoculated at the start of the experiment (day 0) with the fungus. In addition, we established three control treatments to investigate the potential effects of changes in adult density expected to occur in the treated populations. These were: 20 locusts per cage with reductions in density only because of natural control mortality ('control high'); 20 locusts per cage but with insects removed to accompany (sex-specific) mortality in the infected treatment ('control medium'); or just six locusts per cage from the outset to represent a low density population equivalent to that expected in the infected treatment towards the end of the experiment ('control low') (Fig. 1).

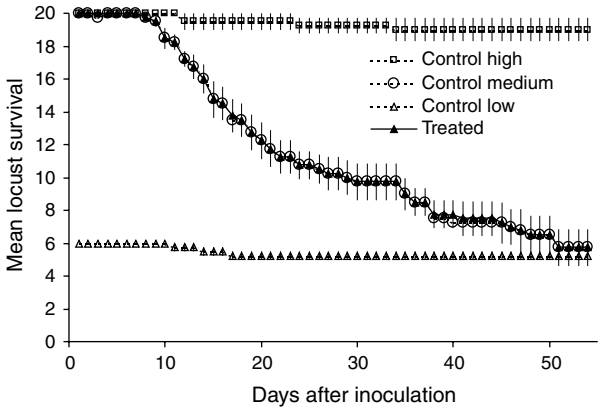

Figure 1 Locust population sizes in the four treatments used in experiment 1. Means of four replicate cages are shown (bars are ±s.e.m.). Note that the population decline in the 'control medium' treatment was principally because of removal of locusts to accompany population size in the 'Infected' treatment. All other changes in population size represent locust mortality.

Mortality in each of the treatments was recorded daily, and qualitative assessments of thermoregulatory behaviour were made twice daily by recording whether locusts were actively basking on top of the mesh shield around the light bulb, or were elsewhere in the cage (Elliot *et al.* 2002).

Following the first observation of mating (*c.* day 25), each cage was supplied with three plastic cups (12 cm deep × 6 cm diameter) filled with moist (i.e. not water-logged) silver sand (from a garden centre) to allow oviposition. These were replaced every 3–5 days, covered with clingfilm and kept in a climate room at 30 ± 1°C. Hatchling locusts were removed from each pot daily and the following colour scores were attributed (Islam *et al.* 1994b):

1 Ground colour uniformly green with no black pattern.
2 Ground colour green with some black markings (<30% of body surface).
3 Ground colour green or olive but with extensive black markings (30–60% body surface) and prominent femoral melanin stripes.
4 Pale ground colour almost obscured by black markings (60–80% of body surface).
5 Ground colour entirely obscured by black markings (>80% of body surface).

These colour scores were used as the primary indicator of phase state (Table 1). In addition, however, subsets of locusts were removed upon hatching to assess their behaviour as they became available. Given that locusts of colour scores 2–5 are expected to show *gregaria*-type behaviour (Islam *et al.* 1994b), we did not select locusts randomly but, rather, compared 29 locusts of colour score 1 against 29 other locusts of the lowest colour scores available (i.e. mostly of score 2 and hence, most likely amongst locusts scoring 2–5 to be *solitaria*, making the comparison conservative). We broadly followed the procedure of Islam *et al.* (1994b)) wherein hatchlings are introduced to the centre of an arena with 50–100 *gregaria* phase hatchlings at one end and various behavioural components are observed. The arena was 35.5 × 15 × 10 cm with a paper grid on the floor to allow the position of locusts to be recorded. Locusts were introduced to the centre of the grid via a syringe and observed through an eyehole in the top of the arena. A Visual Basic program (Microsoft Excel 97 for Windows 95) was used to record behaviours and timings. The following criteria were used to stop an assay: the locust did not move from its starting position in the first 5 min, it made contact with either the left or right wall, it did not reach an end wall within 10 min. When a locust started walking, its start time and eventual stop time were recorded, as were its new grid co-ordinates (*x* and *y*). Locust jumping was recorded together with its co-ordinates upon landing. Locust turns in position of >45°, leg movements, body repositionings, grooming events and crouches were also recorded. From these, the variables shown in Table 2 were calculated for each locust. In the original descriptions of this protocol (e.g. Roessingh *et al.* 1993), these variables were subjected to logistic regression, to give calibrated predictions of the probability that an individual locust was of the solitary phase ('P_{solitary}'). However, we held to an overall comparison of behaviours as we did not have *solitaria* locusts of the same genotype from which to construct the initial logistic regression model. Nevertheless, the behavioural variables from the assay can be related to a biological understanding of phase state.

Experiment 2

Fifth instar gregarious *S. gregaria* were acquired from the same source as above and were held in standard locust cages until fledging. Following this, these young adults were transferred to opaque plastic cages (22 × 15.5 × 11.5 cm^3), ventilated at the top and sides with muslin, with wire mesh inside to allow climbing. Each cage housed seven females and five males – the females were the test animals while the males were present simply to maintain density and

Table 1 Frequencies of colour scores (±s.e.m.) of locust offspring derived from the two experiments ($n = 4$ and 5, respectively). The scale ranges from 1 (all green, indicative of *solitaria* phase state) to 5 (all black, *gregaria* phase state). Frequencies of colour score 1 vs. 2–5 were significantly associated with treatment in each experiment (*G*-tests, $P < 0.001$, see text)

	Hatchling colour score					
Treatment	1	2	3	4	5	Total
Experiment 1						
Infected	14.6 ± 2.5%	6.4 ± 2.9%	7.4 ± 3.8%	6.8 ± 2.0%	64.8 ± 9.8%	1655
Control high	1.4 ± 0.6%	2.5 ± 1.5%	2.4 ± 1.1%	3.6 ± 1.6%	90.2 ± 4.7%	2042
Control medium	1.2 ± 0.4%	2.5 ± 0.6%	2.6 ± 1.0%	10.9 ± 2.0%	82.8 ± 1.5%	1402
Control low	1.3 ± 0.6%	3.3 ± 1.3%	9.3 ± 3.2%	7.7 ± 3.0%	78.5 ± 5.0%	648
Experiment 2						
'Fevered'	13.6 ± 4.6%	13.1 ± 6.0%	1.1 ± 0.9%	9.9 ± 5.5%	62.3 ± 12.6%	780
Control	4.4 ± 1.1%	11.5 ± 3.9%	4.7 ± 2.2%	12.0 ± 5.9%	67.3 ± 4.8%	1031

Table 2 Mean (±s.e.m.) values for the 10 variables recorded from behavioural observations of individual locusts in an observation arena (experiment 1). Data confirm *solitaria* phase state of colour score 1 locust offspring ($n = 29$), compared with offspring of colour scores 2–5 ($n = 29$) ($P = 0.0002$ by non-parametric MANOVA, see text). All frequencies are relative to 10 min observations

Variable	Colour score 1 Mean ± SE	Colour scores 2–5 Mean ± SE
x distance (stimulus = +1)	−0.32 ± 0.14	0.16 ± 0.16
Track straightness	0.94 ± 0.11	1.48 ± 0.18
Track speed (units s^{-1})	0.05 ± 0.01	0.12 ± 0.04
Walking frequency	10.74 ± 2.18	11.42 ± 2.36
Time spent walking (s)	40.24 ± 8.96	44.83 ± 10.18
Jump frequency	0.61 ± 0.35	8.12 ± 4.78
Turns per time	1.92 ± 0.92	1.44 ± 0.66
Leg movement frequency	1.06 ± 0.55	1.37 ± 0.51
Repositioning frequency	0.14 ± 0.10	1.73 ± 0.56
Grooming frequency	1.11 ± 0.58	3.13 ± 1.20

encourage female maturation. There were five replicate pairs of cages which were maintained in a climate room on a 9L : 15D cycle, set at 20 ± 1°C during the dark phase and 44 ± 1°C for 5 h during the middle of the day. The higher temperature setting gave considerable local variation within the climate room, which we exploited by mapping the room to within 1°C with a copper constantan thermocouple (0.125 mm in diameter) linked to a digital thermometer. During the day, the cages were held in random positions at 38–39°C, representing normal locust thermoregulatory temperatures. In addition, for 5 h during the middle of the day [a period consistent with observations in the above experiment and in previous studies (Authors' unpublished data)], one of each of the paired cages was transferred to a random position within the warmer environment such that locusts experienced behavioural fever temperatures of 42–44°C. Following this bout of simulated fever, the cages were placed back at 38–39°C. The procedure was carried out daily for 20 days, effectively covering the sexual maturation phase of the locusts.

Female locusts were then returned to standard locust cages with the fevered and control locusts maintained in separate replicate cages. Ten mature males from the source culture were then added to each of the cages for mating. Oviposition cups filled with sand were placed in the cages and the colour scores of hatchlings assessed as above.

Statistical tests

Comparisons of the basking behaviour of infected and control medium (uninfected) locusts were made by using linear mixed effects models (in R v. 1.5; Crawley 2002, pf. 659) based on the arc-sine transformed proportions of locusts observed to be on the bulb shields (this transformation reduced the heterogeneity of variance). This was carried out for two periods, from day 6 post-inoculation (i.e. after fledging and allowing fever to set in) to day 25 when locusts were sexually mature and from day 26 to 58. This analysis enabled us to account for time as the primary covariate in the repeated observations, and block as the grouping factor. Comparisons between the two treatments were made by F-tests following deletion of treatment from the full models. All tests of frequencies of offspring colour scores were made by tests of independence incorporating G-tests, while differences in the behaviour of the hatchlings was tested by a non-parametric MANOVA (Anderson 2000) of the ten untransformed variables, standardized by their sums, using a Gower metric distance measure.

RESULTS

Experiment 1

Survival curves for the pooled replicated treatments are presented in Fig. 1. These illustrate the locust densities in the four treatments and show very low mortality in control locusts, but a 50% reduction in the infected locust population after *c.* 22 days. All locusts from the infected treatment showed the characteristic red colouration after death, indicative of infection with *Metarhizium* and complete colonization of the cadaver (vs. incomplete colonization – see Elliot *et al.* 2002). The observational studies of locust thermoregulatory behaviour indicated that infected locusts were spending more time basking on the shields surrounding the light bulbs, than the uninfected medium density controls (Fig. 2). During the maturation period (days 6–25), a mean of

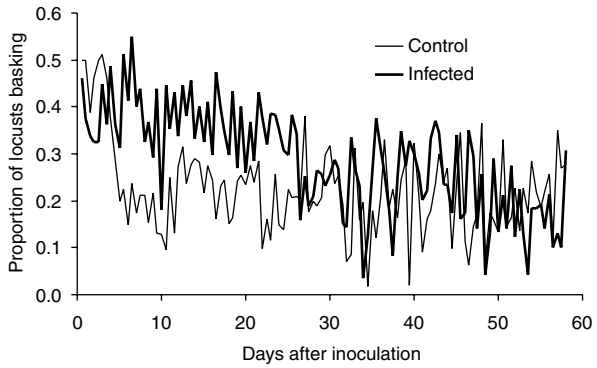

Figure 2 Summary of locust thermoregulatory behaviour in the 'Infected' and 'Control Medium' treatments in experiment 1. Means from the four replicates of twice daily observations of proportions of locusts in the cages observed basking on top of the shields surrounding the light bulbs are shown (N.B. maximum standard error of mean during period of different behaviour, days 6–25, is 0.1.).

0.186 (±0.00163) control medium locusts were recorded on the shields compared with 0.374 (±0.00232) in the infected treatment (linear mixed effects models: $F_{1,8} = 9.70$, $P = 0.0018$). In the post-maturation phase, the infected locusts were no longer fevering (means of 0.143 (±0.00440) for control medium and 0.190 (±0.00601) for infected, with no significant difference, $F_{1,8} = 0.619$, $P = 0.431$). Behaviours of locusts in the other density treatments were similar to control medium so are not shown. The results from the early phase of the disease are similar to those observed on days five and six post-inoculation in a previous experiment utilising similar treatments (e.g. Elliot *et al.* 2002).

Counts and colour scores of locust hatchlings, summed from days 44 to 58, are shown in Table 1. The majority (from 64.8 ± 9.8% to 90.2 ± 4.7%) of hatchlings were of the characteristically *gregaria* colour score 5. However, the infected locusts had more *solitaria* hatchlings (14.6 ± 2.5% colour score 1) than any of the other treatments (from 1.2 ± 0.4% to 1.4 ± 0.6% colour score 1). A 2 × 4 test of independence (colour scores 1 vs. 2–5) showed these frequencies to be associated with treatment ($P \ll 0.001$ as $G = 323.0$ is greater than $\chi^2_{0.001[3]} = 16.266$). In addition, a slight density effect was apparent in the control groups in the proportion of category 5 hatchlings (Table 1; 2 × 3 test of independence, colour scores 1–4 vs. 5: $P < 0.001$ as $G = 76.69$ is greater than $\chi^2_{0.001[2]} = 13.816$).

The behavioural assays showed quite clear differences between the behaviour of colour score 1 hatchlings vs. hatchlings with scores of 2–5 (Table 2; non-parametric MANOVA, $F_{1,56} = 35.95$, $P = 0.0002$,). The direction of the differences were all consistent with known differences in behaviour attributable to phase state (Roessingh *et al.* 1993; Islam *et al.* 1994a,b; Boua *et al.* 1995), confirming that colour score 1 individuals were *solitaria* nymphs, and colour scores 2–5 were *gregaria*.

Experiment 2

The colour scores of hatchlings from the simulated fever regime ('Fever') and the control regime are shown in Table 1. The pattern was broadly similar to that for infected and uninfected locusts from the first experiment (also Table 1), in that the 'Fevered' locusts produced more solitary, colour score 1 offspring (13.6 ± 4.6%) than did the controls (4.4 ± 1.1%). A 2 × 2 test of independence (colour scores 1 vs. 2–5) showed these frequencies to be associated with treatment ($P \ll 0.001$ as $G = 1388.7$ is greater than $\chi^2_{0.001[1]} = 10.828$).

DISCUSSION

The principal aim of this study was to examine the effect of *M. anisopliae* var. *acridum* infection in adult *gregaria* locusts, on the phase state of their offspring. Contrary to the pattern expected from the density-dependent prophylaxis hypothesis and adaptive maternal effects, *Metarhizium* infection increased solitarization. This is confirmed by the production of 14.6% offspring with *solitaria* colouration from infected *gregaria* parents, vs. <2% from their uninfected counterparts (Table 1). In previous work (e.g. Islam *et al.* 1994b), <5% of the offspring of crowd-reared parents were of colour score 1 (i.e. similar to the controls here) unless mothers were isolated during oviposition. The behavioural assays showed typical *solitaria* behaviour in score 1 vs. other score (the lowest available) locusts, confirming their phase state (Table 2). Given that parent locusts in the current study had visual and olfactory stimuli from other locusts in the same climate room, such a shift in phase state is quite striking.

The base state of locusts is a *solitaria* phenotype and it is the parental phase and their experience of crowding which triggers the gregarization of offspring. This process involves mechanical, visual and chemical cues, the former being the most important in eliciting behavioural gregarization (Roessingh *et al.* 1998; Hägele & Simpson 2000; Simpson *et al.* 2001). The mother releases a gregarizing agent from the reproductive tract (Hägele & Simpson 2000) into the egg foam (McCaffery *et al.* 1998) from where it enters the eggs and affects development. One possible explanation for the shift towards solitarization, then, is that the declining densities in the infected treatments resulted in the adults receiving fewer gregarizing stimuli during reproduction (i.e. an indirect ecological effect of infection via population reduction of infected parents). However, this is discounted by the low numbers of solitary offspring resulting from the three control treatments that were designed to incorporate the influence of density (Fig. 1 & Table 1). Similarly, vertical transmission of *Metarhizium* has never been recorded in acridids so can presumably be discounted as a factor. We are left, therefore, with infection itself and/or the host behavioural fever response, as proximate factors responsible for the observed effect. Given the results of the second experiment, in which simulated behavioural fever was seen to increase the production of solitary offspring even in the absence of infection, it appears that it is the elevated body temperatures associated with behavioural fever which are, in large part, responsible for the observed solitarization.

Exactly how fever temperatures induce such effects is unclear. It is possible that the production or action of the gregarizing factor, or its delivery to the eggs, may have been directly affected by the elevated body temperature (Fig. 2). In some other systems, stress factors that compromise the activity of heat shock proteins may reveal an organism's underlying phenotypic variability (Rutherford & Lindquist 1998; Queitsch *et al.* 2002). Thus, there might be mechanisms through which fever in locusts affects heat shock proteins, so revealing a range of phenotypes (in this case,

phase state). More generally, whilst fever has been shown to provide survival benefits (Elliot *et al.* 2002), such an increase in body temperature above the normal set point is expected to carry costs (Kluger *et al.* 1998). Typically these may be manifested as direct energetic costs (Muchlinski 1985; Kluger *et al.* 1998), or may be mediated via other traits or processes such as feeding efficiency, growth rate and escape from predation (Boorstein & Ewald 1987; Lefcort & Eiger 1993; Forsman 1999). In this context, the transgenerational effect on host phenotype we observe here is quite surprising, and highlights the potential complexities in exploring trade-offs and correlations between life-history traits (c.f. Kraaijeveld & Godfray 1997).

Overall, this study suggests a novel mechanism for environmental effects on locust phase state. Given the greater vulnerability of *solitaria* locusts to pathogens (Wilson *et al.* 2002), our results also suggest a somewhat counter-intuitive response to natural enemy attack. The fitness consequences of this effect on offspring phenotype are, however, unclear. For example, the difference in total numbers of offspring of colour score 2–5 (i.e. *gregaria* and so less vulnerable to pathogens) between comparable treatments is actually minor (1433 from the infected parents vs. 1385 from the control medium parents). Thus the *solitaria* offspring could be considered as a bonus in terms of parental inclusive fitness. The effect of the fever treatment is more striking, with a reduction in total offspring and an increase in *solitaria*. Hence, although this implicates and suggests a cost of fever in the overall response, it appears that there may be an interaction with infection itself [infection has been shown to affect rate of sexual maturation and initial reproductive output of gregarious locusts (Blanford & Thomas 2001)]. Moreover, although the increased production of *solitaria* can be viewed as a cost in the context of the density-dependent prophylaxis hypothesis, the behaviour of solitary locusts, in which they typically disaggregate and move away from other individuals, could be beneficial in terms of reducing risk of infection. Therefore, it is possible that the production of a higher proportion of solitary locusts is an adaptive transgenerational response targeted at transmission, rather than resistance; a result that adds complexity to the interpretation of the density-dependent prophylaxis hypothesis. Finally, the possibility remains that the phenomenon may be adaptive to the pathogen (although the fact that it can be generated in the absence of the pathogen makes this unlikely).

Whatever the exact processes involved, a reduction in the tendency of locusts to aggregate in swarms, coupled with an increase in their vulnerability to pathogens, represent potentially useful side effects of infection which could increase the scope for biological control of locusts using *Metarhizium*-based technologies. More generally, the study illustrates how ecological and evolutionary interpretation of resistance is complicated when multiple factors are considered. Although this may seem obvious, many studies tend to reduce systems down to their basic components to make research problems more tractable. By considering behaviour, environment and transgenerational effects, the current study adds to a growing body of literature (e.g. Bohannan & Lenski 2000; Ackermann *et al.* 2001; Ferguson & Read 2002; Yourth *et al.* 2002; Blanford *et al.* 2003; Thomas *et al.* 2003), which challenge the reductionist approach and indicate the importance of interacting factors and some element of condition dependency in understanding the ecology and evolution of resistance.

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