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

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.

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; Bouaı¨chi 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 hatching will behave just like a gregaria after a few hours of contact with other locusts (Bouaıchi 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 (Lomet 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 solitary locusts are more susceptible to pathogens such as Metarhizium, we might expect an increase in gregarization of locust offspring produced by an infected solitary 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).

METH ODS

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 × 10⁴ 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 (Elliott 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. Hatching 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 showgregaria-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 transferred 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 (Psolitary). 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 cm³), 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) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment       | 1               | 2               | 3               | 4               | 5               | Total           |
| 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             |
| 'Fevered'       | 14.5 ± 2.6%     | 13.1 ± 6.0%    | 1.1 ± 0.9%     | 9.9 ± 5.5%     | 62.3 ± 12.6%   | 780             |
| Control         | 13.1 ± 4.6%     | 11.5 ± 3.9%    | 4.7 ± 2.2%     | 12.0 ± 5.9%    | 67.3 ± 4.8%    | 1031            |

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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 *S. gregaria* 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.

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

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 *M. bactharchium* 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](image_url)
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 < 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 < 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; Quetsch 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; Youth 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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REFERENCES


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