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Crowded locusts produce hatchlings vulnerable to fungal attack

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Transgenerational effects of parental experience on offspring immunity are well documented in the vertebrate literature (where antibodies play an obligatory role), but have only recently been described in invertebrates. We have assessed the impact of parental rearing density upon offspring disease resistance by challenging day-old locust hatchlings (*Schistocerca gregaria*) from either crowd- or solitary-reared parents with the fungal pathogen *Metarhizium anisopliae* var. *acridum*. When immersed in standardized conidia suspensions, hatchlings from gregarious parents suffered greater pathogen-induced mortality than hatchlings from solitary-reared parents. This observation contradicts the basic theory of positive density-dependent prophylaxis and demonstrates that crowding has a transgenerational influence upon locust disease resistance.

Keywords: insect immunity; maternal effects; fungal pathogens

1. INTRODUCTION

Although vertebrate offspring can inherit parental immune function through antibodies (Grindstaff *et al.* 2003), mechanisms of disease resistance in invertebrates have been thought to depend less upon prior experience (e.g. Roitt 1997). However, recent studies have found that invertebrate parents exposed to pathogens may produce offspring with increased resistance; this phenomenon is termed 'transgenerational immune priming' (Little *et al.* 2003; Sadd *et al.* 2005; Moret 2006; Sadd & Schmid-Hempel 2007). The ways in which invertebrate offspring resistance may relate to aspects of parental experience independent of pathogen pre-exposure, such as population density or rearing conditions, have not been systematically investigated.

Because the probability of encountering disease agents may increase with population density (McCallum *et al.* 2001), increased investment in immune defence is sometimes observed when hosts are crowded. This is known as density-dependent prophylaxis (DDP) (Wilson & Reeson 1998). In insect species exhibiting DDP, resistance to pathogen attack correlates positively with host population density (Wilson & Cotter 2008).

In accordance with the prediction of DDP, adult desert locusts (*Schistocerca gregaria* Forskål) from a gregarious culture were found to be more resistant to fungal challenge than solitary-reared equivalents (Wilson *et al.* 2002). Locusts exhibit density-dependent phase polyphenism, having the potential to exist in either the 'solitarious' or 'gregarious' phase depending on population density and experience of crowding, and it was proposed that these different immune responses are part of the suite of adaptive traits accompanying phase differentiation. However, because standard gregarious culture conditions are highly conducive to pathogen growth (Charnley *et al.* 1985), differential immune defence may be explained by the death of low-resistance gregarious individuals prior to testing, or induction of enhanced resistance through exposure to pathogens, rather than by phase-specific effects *per se*. Thus, the relative contributions of ontogeny and maternal effects to locust disease resistance remain unclear.

Locust phase state changes within an individual's lifetime in response to crowding. Behaviour and other physical and physiological traits also change as a function of parental rearing density due to chemically mediated maternal effects (Miller *et al.* 2008; Pener & Simpson 2009). Hatchlings of gregarious parents are more likely be in densely populated environments than those from solitarious parents (e.g. Bouaichi & Simpson 2003), and gregarious-parent hatchlings of *S. gregaria* are larger than solitarious-parent hatchlings and survive longer when starved (Uvarov 1966). The role, if any, of these or other transgenerational effects upon hatchling disease resistance has not been explored. Infection of parents with a fungal pathogen can affect offspring behaviour and coloration (Elliot *et al.* 2003), but there have been no assessments of locust hatchling immunity as a function of parental rearing density.

The aim of this study was to determine whether there is a transgenerational effect of parental rearing density upon offspring pathogen resistance in the desert locust (*S. gregaria*). Locust hatchlings (on the day of eclosion) were challenged by immersion into known concentrations of viable conidia of the acridid-specific fungal pathogen *Metarhizium anisopliae* var. *acridum* (a species used effectively as a locust biocontrol agent in the field; Lomer *et al.* 1997). To avoid any potentially confounding effects of hatchling culture conditions, solitarious and gregarious eggs were removed from their respective cultures and group-hatched in identical circumstances.

2. MATERIAL AND METHODS

(a) *Insects*

Locusts (*S. gregaria*) from solitarious and gregarious cultures (from the same genetic background) were reared using standard techniques as detailed in Roessingh *et al.* (1993) at the Department of Zoology, University of Oxford, United Kingdom.

(b) *Pathogen handling*

M. anisopliae var. *acridum* (IMI 330189) conidia were obtained as a technical powder from CABI Bioscience and uniform aqueous suspensions were produced in a sonicator (stock solution: 50 mg conidia powder in 5 ml HPLC-grade water, sonicated for 10 min). Conidia concentration in suspension was quantified using a Neubauer haemocytometer and viability was determined by quantifying conidium germination on Sabouraud dextrose agar plates (Oxoid) after incubation at 25°C for 20 h (Goettel & Inglis 1997).

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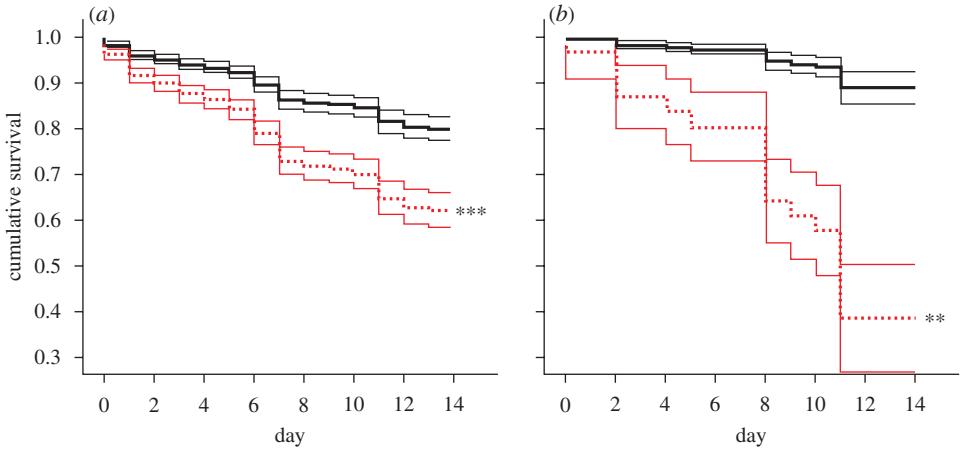

Figure 1. (*a*,*b*) Pathogen challenge affects survivorship of hatchlings from both (*a*) solitarious and (*b*) gregarious parents. Solid black lines indicate control treatments and dashed red lines indicate pathogen treatments. Thin, flanking lines show standard errors. Fungal pathogens increase mortality in both groups, but the impact upon gregarious-parent insects is significantly greater (see table 1). ($n = 51$ gregarious; $n = 228$ solitarious; $^{**}p < 0.01$; $^{***}p < 0.001$.)

(c) *Pathogen challenge of hatchlings from gregarious and solitarious parents*

Freshly enclosed (>24 h old) hatchlings originating from gregarious ($n = 51$) and solitarious ($n = 228$) cultures were dipped individually (using fine forceps) into either distilled water or freshly sonicated aqueous suspensions of *M. anisopliae* var. *acridum* containing 5×10^3 conidia ml^{-1}. Hatchlings were then housed in groups of 7–21 insects at 25°C (±0.5°C) in a total of 22 plastic containers (11 cm × 17 cm × 5 cm) into which fresh wheatgrass was placed daily. Treated and control insects were placed in different containers to prevent horizontal pathogen transmission, and insects from given solitarious mothers were placed in the same containers to assess parental effects on resistance. Survivorship was assessed daily for 14 days. Dead insects were removed immediately from group environments and random samples of these ($n = 50$ inoculated; $n = 30$ control, split equally between solitarious and gregarious colonies) were placed into humidity chambers to check for sporulation.

(d) *Statistical analysis*

Data were analysed in SPSS 14 (SPSS Inc.). Death rates were modelled using Cox regression survivorship analysis where pathogen presence was cast as a simple time-dependent covariate equal to (log(time) × (pathogen concentration)).

3. RESULTS

Pathogen exposure significantly reduced survivorship in populations of hatchlings from both solitarious and gregarious parents (Wald = 12.739, d.f. = 1, $p < 0.001$; Wald = 7.381, d.f. = 1, $p = 0.007$, respectively; figure 1). Fewer solitarious-parent hatchlings died owing to pathogen challenge than hatchlings from gregarious parents (61 and 38% survival at day 14, respectively; figure 1, table 1). The survivorship curves of control hatchlings from solitarious and gregarious parents were not significantly different (Wald = 0.221, d.f. = 1, $p = 0.638$; black lines, figure 1), and control mortality did not differ as a function of particular housing container (Wald = 7.56, d.f. = 10, $p = 0.672$). Furthermore, hatchlings from solitarious parents did not react differently to the pathogen challenge depending upon the identity of their solitarious mother (Wald = 9.10, d.f. = 7, $p = 0.246$). Sporulation, indicating mycosis, was observed in 94 per cent of inoculated individuals who died prior to trial termination and in none of the tested control (water-immersed) insects.

Table 1. Cox survivorship analysis of fungus-treated locust hatchlings from solitarious and gregarious parents. ($^{*}p<0.05$; $^{***}p<0.001$.)

	Wald statistic	d.f.	*p*	odds ratio
pathogen	13.120	1	<0.001***	3.388
phase of hatchling parent (solitarious or gregarious)	2.440	1	0.118	0.364
pathogen × phase	4.793	1	0.029*	5.288

4. DISCUSSION

Hatchlings from crowded parents were more vulnerable to fungal attack than hatchlings from isolated (solitarious) parents (figure 1, table 1). Although previous studies have examined insect immunity in offspring of pathogen-challenged parents (Moret 2006; Sadd & Schmid-Hempel 2007), parental population density has not previously been shown to affect hatchling pathogen resistance.

Fungal inoculation by immersion provides dosages proportional to insect surface area: larger insects receive smaller dosages per unit weight because surface area scales more slowly than volume with increasing size. Considering the significantly larger size of *S. gregaria* hatchlings from gregarious relative to solitarious parents (Uvarov 1966; Injeyan & Tobe 1981; Tanaka & Maeno 2008; Pener & Simpson 2009), their comparatively poor pathogen resistance runs counter to expectation based upon dosage, and is particularly striking.

Despite the increased likelihood of hatching into high-density populations, gregarious-mother hatchlings were less resistant to pathogen challenge; this is contrary to the result predicted by DDP (Wilson & Reeson 1998; Wilson *et al.* 2002). The high relative resistance of solitarious hatchlings is nevertheless in accord with data from Wilson *et al.* (2003), in which five of six solitarious lepidopteran species showed increased correlates of immune

function (haemocyte count and phenoloxidase activity) relative to phylogenetically matched gregarious species. Futhermore, Pie *et al.* (2005) found that termites at high and low population densities had comparable resistance to a fungal challenge, suggesting that eusocial insects may not conform to the DDP hypothesis.

The present results support the contention that DDP is best understood in a broader context of both within- and between-group infection rates; the risk of pathogen exposure may be minimal for group members whose between-group infection risk is low enough to compensate for potentially high transmission within the group (Wilson *et al.* 2003). Indeed, the recent application of 'percolation theory' to disease spread demonstrates that the spatial clustering of host individuals (as in gregarious locust populations) can reduce the ability of natural enemies to move between resources on a larger scale (Reynolds *et al.* 2008; Wilson 2009).

In addition to variation in exposure to disease agents, distinct life-history and survival strategies may also underlie the observed reduction in the pathogen resistance of hatchlings from crowded parents. Because solitarious nymphs take longer to reach their reproductive potential (Pener & Yerushalmi 1998), they are subject to greater time-dependent mortality risks (including pathogen exposure), which may justify parental investment in offspring immune function. Meanwhile, gregarious locusts are adapted for migration—for example through their larger size, longer wings, increased activity levels, greater fat storage and other phase differences (Pener & Simpson 2009)—and, therefore, may have fewer energetic or nutritional resources available for immune defence. Accordingly, resource allocation trade-offs at the maternal and/or offspring levels may contribute to limited pathogen resistance in insects from gregarious parents. Future studies may investigate how other aspects of the parental environment affect offspring immunity and whether parents trade off their own resistance against that of their progeny.

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Bouaichi, A. & Simpson, S. J. 2003 Density-dependent accumulation of phase characteristics in a natural population of the desert locust *Schistocerca gregaria*. *Physiol. Entomol.* **28**, 25–31. (doi:10.1046/j.1365-3032.2003.00317.x)

Charnley, A. K., Hunt, J. & Dillon, R. J. 1985 The germ-free culture of desert locusts, *Schistocerca gregaria*. *J. Insect Physiol.* **31**, 477–485. (doi:10.1016/0022-1910(85)90096-4)

Elliot, S. L., Blanford, S., Horton, C. M. & Thomas, M. B. 2003 Fever and phenotype: transgenerational effect of disease on desert locust phase state. *Ecol. Lett.* **6**, 830–836. (doi:10.1046/j.1461-0248.2003.00487.x)

Goettel, M. S. & Inglis, G. D. 1997 Fungi: hyphomycetes. In *Manual of techniques in insect pathology* (ed. L. A. Lacey), pp. 213–249. New York, NY: Academic Press.

Grindstaff, J. L., Brodie, E. D. & Ketterson, E. D. 2003 Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proc. R. Soc. Lond. B* **270**, 2309–2319. (doi:10.1098/rspb.2003.2485)

Injeyan, H. S. & Tobe, S. S. 1981 Phase polymorphism in *Schistocerca gregaria*: reproductive parameters. *J. Insect Physiol.* **27**, 97–102. (doi:10.1016/0022-1910(81)90115-3)

Little, T. J., O'Connor, B., Colegrave, N., Watt, K. & Read, A. F. 2003 Maternal transfer of strain-specific immunity in an invertebrate. *Curr. Biol.* **13**, 489–492. (doi:10.1016/S0960-9822(03)00163-5)

Lomer, C. J., Prior, C. & Kooyman, C. 1997 Development of *Metarhizium* sp. for the control of grasshoppers and locusts. *Memoirs Entomol. Soc. Canada*, 265–286.

McCallum, H., Barlow, N. & Hone, J. 2001 How should pathogen transmission be modelled? *Trends Ecol. Evol.* **16**, 295–300. (doi:10.1016/S0169-5347(01)02144-9)

Miller, G. A., Islam, M. S., Claridge, T. D. W., Dodgson, T. & Simpson, S. J. 2008 Swarm formation in the desert locust *Schistocerca gregaria*: isolation and NMR analysis of the primary maternal gregarizing agent. *J. Exp. Biol.* **211**, 370–376. (doi:10.1242/jeb.013458)

Moret, Y. 2006 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proc. R. Soc. B* **273**, 1399–1405. (doi:10.1098/rspb.2006.3465)

Pener, M. P. & Simpson, S. J. 2009 *Locust phase polyphenism: an update*. *Advances in Insect Physiology*, vol. 36. San Diego, CA: Academic Press.

Pener, M. P. & Yerushalmi, Y. 1998 The physiology of locust phase polymorphism: an update. *J. Insect Physiol.* **44**, 365–377. (doi:10.1016/S0022-1910(97)00169-8)

Pie, M., Rosengaus, R., Calleri, D. I. & Traniello, J. 2005 Density and disease resistance in group-living insects: do eusocial species exhibit density-dependent prophylaxis? *Ethol. Ecol. Evol.* **17**, 41–50.

Reynolds, A., Sword, G., Simpson, S. & Reynolds, D. 2008 Predator percolation, insect outbreaks and phase polyphenism. *Curr. Biol.* **19**, 20–24. (doi:10.1016/j.cub.2008.10.070)

Roessingh, P., Simpson, S. J. & James, S. 1993 Analysis of phase-related changes in behavior of desert locust nymphs. *Proc. R. Soc. Lond. B* **252**, 43–49. (doi:10.1098/rstb.1993.0044)

Roitt, I. M. 1997 *Essential immunology*. Oxford, UK: Blackwell Publishing.

Sadd, B., Kleinlogel, Y., Schmid-Hempel, R. & Schmid-Hempel, P. 2005 Trans-generational immune priming in a social insect. *Biol. Lett.* **1**, 386. (doi:10.1098/rsbl.2005.0369)

Sadd, B. M. & Schmid-Hempel, P. 2007 Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Curr. Biol.* **17**, 1046–1047. (doi:10.1016/j.cub.2007.11.007)

Tanaka, S. & Maeno, K. 2008 Maternal effects on progeny body size and color in the desert locust, *Schistocerca gregaria*:

examination of a current view. *J. Insect Physiol.* **54**, 612–618. (doi:10.1016/j.jinsphys.2007.12.010)

Uvarov, B. P. 1966 *Grasshoppers and locusts: a handbook of general acridology*, vol 1. London, UK: Cambridge University Press.

Wilson, K. 2009 Evolutionary ecology: old ideas percolate into ecology. *Curr. Biol.* **19**, 21–23. (doi:10.1016/j.cub.2008.11.049)

Wilson, K. & Cotter, S. C. 2008 Density-dependent prophylaxis in insects. In *Phenotypic plasticity of insects: mechanisms and consequences* (eds D. W. Whitman & T. N. Ananthakrishnan), pp. 381–420. Enfield, NH: Science Publishers.

Wilson, K., Knell, R., Boots, M. & Koch-Osborne, J. 2003 Group living and investment in immune defence: an interspecific analysis. *J. Anim. Ecol.* **72**, 133–143. (doi:10.1046/j.1365-2656.2003.00680.x)

Wilson, K. & Reeson, A. F. 1998 Density-dependent prophylaxis: evidence from lepidoptera–baculovirus interactions? *Ecol. Entomol.* **23**, 100–101. (doi:10.1046/j.1365-2311.1998.00107.x)

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. *Proc. Natl Acad. Sci. USA* **99**, 5471–5475. (doi:10.1073/pnas.082461999)