Journal of Animal Ecology 2003
72, 133–143

Group living and investment in immune defence: an interspecific analysis

KENNETH WILSON, ROBERT KNELL*, MICHAEL BOOTS and JANE KOCH-OSBORNE
Institute of Biological Sciences, University of Stirling, Stirling, FK9 4LA, UK

Summary

1. Since parasite transmission is often density-dependent, group living is normally thought to lead to an increased exposure to parasitism. As a consequence, it is predicted that animals living in groups will invest more resources (energy, time, risk, etc.) in parasite defence than those living solitarily.
2. We tested this prediction by measuring basal immune parameters in the larvae of 12 species of Lepidoptera, grouped into six phylogenetically matched species-pairs, each comprising one solitary feeding and one gregariously feeding species.
3. Contrary to expectation, the solitary species in all six species-pairs had higher total haemocyte counts than the gregarious species, and in five out of six species-pairs the solitary species also exhibited higher phenoloxidase activity. Both measurements were positively correlated with each other and with the magnitude of the cellular encapsulation response.
4. The relationship between infection risk and group living was investigated with a dynamic, spatially explicit, host–pathogen model. This shows that when individuals aggregate in groups, the *per capita* risk of infection can be reduced if the lower between-group transmission more than compensates for the higher within-group transmission.
5. We conclude that the expectation that group living always leads to increased exposure to pathogens and parasites is overly simplistic, and that the specific details of the social system in question will determine if there is increased or decreased exposure to infection.

Key-words: aggregation, costs of sociality, group living, immunocompetence, infection, Lepidoptera, pathogen.

Journal of Animal Ecology (2003) **72**, 133–143

Introduction

In his review paper on the evolution of social behaviour, Alexander (1974) wrote: 'Although I believe not yet tested, the prediction is compelling that group-living animals will either be plagued more heavily with parasites and diseases than their solitary-living close relatives, or they will be plagued with greater expense of time and energy, and greater risk, in reducing the attacks of such organisms.' The logic behind this argument is simple: since parasite transmission is usually positively density-dependent (Anderson & May 1979; McCallum, Barlow & Hone 2001) and animals living in groups will generally experience higher local densities of conspecifics than those living solitarily, they will also experience higher rates of parasite transmission (Freeland 1979; Dobson 1988; Coté & Poulin 1995). A further reason why the impact of parasites might be greater in group-living animals is that higher rates of horizontal transmission (and multiple infections) are associated with the evolution of increased virulence, and so if group living enhances these factors then the parasites of social species may be more virulent, as well as more common, than those of solitary species (Møller *et al*. 2001). Empirically, the relationship between group size and parasitism has been examined many times within species, and a recent meta-analysis indicated that there were consistent positive correlations

Correspondence: Dr Kenneth Wilson, Institute of Biological Sciences, University of Stirling, Stirling, FK9 4LA, U.K. Tel. +44 1786467807, Fax: +44 1786870706, E-mail: ken.wilson@stirling.ac.uk
*Present address: School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, United Kingdom.

between group size and parasitism rates for parasites transmitted by close contact or by a faecal–oral route, whereas the correlations for mobile parasites that actively seek out their hosts (e.g. flies) were consistently negative (Coté & Poulin 1995).

In contrast to the large number of these intraspecific studies, only a few interspecific investigations into group living and parasitism have been made and these present an equivocal picture (Davies *et al*. 1991; Poulin 1991a,b; Poiani 1992; Ranta 1992; Watve & Sukumar 1995). For example, although the prevalence of malaria was positively correlated with sleeping-group size across 31 species of Amazonian primates (Davies *et al*. 1991), among 12 species of Indian mammal the intensity of parasitism by directly transmitted parasites was significantly higher in solitary species than in gregarious species (Watve & Sukumar 1995). Unfortunately, interpretation of the results of both of these studies is complicated by a number of factors, particularly the confounding influences of host and parasite phylogeny (Harvey & Pagel 1991). This is important because spurious correlations are easily generated in comparative analyses when they include groups of related species that share common traits through shared ancestry, rather than convergent evolution (Harvey & Pagel 1991).

Four studies have examined the idea that group-living animals would need to invest more resources (e.g. time, energy, risk, etc.) into defending themselves against parasites and pathogens. First, Hochberg (1991) argued that gregarious Lepidopteran species invest relatively more in disease resistance mechanisms than solitary species. To support this suggestion, he used data gleaned from the literature to show that, in laboratory bioassays using baculoviruses, larvae of gregariously feeding species exhibited an age-related increase in virus resistance that was not observed in solitary-feeding species. Although these results are consistent with Hochberg's hypothesis, there are a number of problems with the analysis, as discussed by the author in the original publication. The analysis did not take account of host phylogeny (Harvey & Pagel 1991) and, because data from the literature were used, it was not possible to control for several confounding variables such as host diet, rearing conditions and coevolved resistance mechanisms. It has long been recognized that host susceptibility to pathogens is influenced by environmental factors, such as diet, temperature, humidity and radiation (Watanabe 1987). The origin of the pathogens is also important because there may be considerable variation both within and between pathogen species in their virulence to potential hosts (Tanada & Fuxa 1987; Gomez, Moscardi & Sosa-Gomez 1999). Co-evolved interactions between pathogens and their hosts may confuse the picture still further, since a host that appears relatively susceptible when one pathogen isolate, strain or clone is used may seem relatively resistant should an alternative one be chosen (Lively & Dybdahl 2000).

A second investigation into group living and immune defence examined the correlation between nesting colony size and response to immune challenge in a study of 13 species of birds from the family Hirundinidae (Møller *et al*. 2001). Immune function was measured as the response to phytohaemagglutinin (which stimulates a T-lymphocyte response), and the response to sheep red blood cells (which stimulate a B- and T-lymphocyte response). Both of these measures of immunocompetence were found to be positively correlated with nesting colony size, even after phylogeny was controlled for. Finally, two studies have examined whether group size is related to counts of circulating leucocytes in primates (Nunn, Gittleman & Antonovics 2000; Semple, Cowlinshaw & Bennett 2002), and both found that group size was not an important determinant of leucocyte count. Thus, the evidence published so far is equivocal, with some support for the idea that social species invest more in immune defences, but also some cases where there seems to be no relationship.

THEORETICAL INVESTIGATIONS OF GROUP LIVING AND PARASITISM

In contrast to the attention that empiricists have paid to the issue of the relationship between group living and parasitism, there has been little theoretical interest in this issue. One exception is a recent modelling study by Watve & Jog (1997), which suggests that decreased disease transmission between groups of hosts may, in fact, more than compensate for increased transmission within groups, leading to a reduced overall risk of infection for clustered host individuals relative to dispersed ones. This model is epidemiological and therefore makes a number of assumptions that make it difficult to assess whether it can be applied to host–pathogen interactions in wildlife hosts. In particular, the model assumes a fixed host population size, which is a reasonable assumption for epidemiological models of many modern human diseases, but is not appropriate to dynamical ecological systems. It also assumes that the population is arranged in clusters of a fixed size and that infection probability decreases with distance between the clusters. There are no births, and the course of the epidemics continues until there are no susceptible individuals or the pathogen is lost from the host population. Crucially therefore the risk of infection in the Watve and Jog model is a product of both the rate of transmission of the disease through the population and the length of time that the pathogen persists.

OBJECTIVES

The present study has two objectives. First, we follow Hochberg's lead and examine the relationship between group living and investment in disease resistance mechanisms in Lepidopteran larvae, and second, we clarify

Table 1. Summary of species used, diet and immune function parameters

Species	Pair	Family	Lifestyle	Diet	*n*	Weight[a]	THC[b]	PO activity[c]
Polygonia c-album (L.)	1	Nymphalidae	Solitary	Nettle	25	0·27 ± 0·07	5401 ± 2258	0·93 ± 0·32
Ianachis io (L.)	1	Nymphalidae	Gregarious	Nettle	30	0·32 ± 0·08	2727 ± 1005	0·55 ± 0·35
Vanessa atalanta (L.)	2	Nymphalidae	Solitary	Nettle	41	0·42 ± 0·09	4155 ± 1212	0·77 ± 0·35
Aglais urticae (L.)	2	Nymphalidae	Gregarious	Nettle	20	0·07 ± 0·03	1525 ± 1296	0·68 ± 0·25
Pieris napi (L.)	3	Pieridae	Solitary	Cabbage	30	0·08 ± 0·03	5405 ± 2977	0·83 ± 0·59
Pieris brassicae (L.)	3	Pieridae	Gregarious	Cabbage	30	0·29 ± 0·09	4089 ± 1263	1·54 ± 0·72
Cerura vinula (L.)	4	Notodontidae	Solitary	Willow	23	1·35 ± 0·34	7019 ± 3127	0·81 ± 0·20
Phalera bucephala (L.)	4	Notodontidae	Gregarious	Willow	17	0·76 ± 0·39	3166 ± 1271	0·66 ± 0·31
Antheraea pernyi (Guénerin-Méneville 1855)	5	Saturniidae	Solitary	Beech	30	1·98 ± 0·32	4155 ± 1212	0·96 ± 0·55
Aglia tau (L.)	5	Saturniidae	Gregarious	Beech	25	0·93 ± 0·39	2223 ± 1067	0·38 ± 0·37
Spodoptera frugiperda (J.E. Smith 1797)	6	Noctuidae	Solitary	Semisynthetic diet	22	0·25 ± 0·04	16 585 ± 6349	1·26 ± 0·44
Spodoptera exempta ((Walker) Zimmerman 1958)	6	Noctuidae	Gregarious	Semisynthetic diet	60	0·34 ± 0·06	13 160 ± 5237	0·88 ± 0·29

[a]Mean weight in g ± SD.
[b]Total haemocyte count (mean number per µl ± SD).
[c]mean log phenoloxidase units per mg protein ± SD.

the predicted relationship between group size and infection risk for temporally and spatially dynamic host–pathogen systems.

Experimental methods

STUDY INSECTS

The 12 species used in the main experiment are listed in Table 1. Each species-pair involves two species that belong to the same family and were reared on the same host plant at approximately the same time. In each case, the solitary species lays its eggs singly or the larvae disperse from the egg mass soon after hatching, whereas the gregarious species lays its eggs in masses and the larvae tend to aggregate. The choice of species was largely governed by availability. Most of the species were acquired as eggs or as newly emerged larvae from *Worldwide Butterflies* (Compton House, Sherbourne, Dorset, DT9 4QN, UK) between May and July 1999 or between April and June 2000. The *Spodoptera* larvae were obtained from cultures that are maintained in our laboratory.

Shortly after emergence, larvae were separated into groups of 10 and reared in 18 × 12 cm plastic tubs. Leaves of the appropriate food plant (or artificial wheatgerm-based diet) were provided *ad libitum*, and frass and old food was removed every 2 days. Larvae were kept at room temperature (23 ± 2 °C), with natural lighting (approximately 18 h light: 6 h dark).

MEASURING IMMUNE PARAMETERS

Rather than studying susceptibility to pathogens directly, we compared basal levels of investment in immune defence using total haemocyte count (THC) and phenoloxidase (PO) activity as our measures of insect immune function. By using matched pairs of solitary and gregarious species from the same family, assayed in similar environments, we were able to control both for host phylogeny and for any influence of host diet or rearing conditions. Moreover, by assaying aspects of the host's immune system, rather than pathogen resistance *per se*, we avoided potential problems associated with parasite phylogeny and host–parasite coevolution. We focused on haemocytes in this study because these are the effector cells of the insect immune system, responsible for mediating defence reactions against both small pathogens (including bacteria, fungi and protozoa) and large, multicellular parasites (including nematodes and parasitoids) (Ratcliffe & Rowley 1987; Gupta 1991; Gillespie, Kanost & Trenczek 1997). The haemocytes are also a major site for the production of the soluble proteins and enzyme cascades that make up the humoral arm of the insect immune system (Gupta 1991; Ratcliffe 1993; Pech & Strand 1995). Whilst some authors distinguish between different haemocyte types, usually on the basis of gross morphology, a number of studies have shown that the overall density of haemocytes in the haemolymph (total haemocyte count) provides a good proxy for the density of immune-active haemocytes present (e.g. Eslin & Prévost 1996, 1998; Kraaijeveld, Limentani & Godfray 2001). Phenoloxidase is an important enzyme in insect immune defence and has been implicated in non-self recognition, as well as in resistance to a range of parasites and pathogens (Rowley, Brookman & Ratcliffe 1990; Ourth & Renis 1993; Hagen, Grunewald & Hamm 1994; Hung & Boucias 1996; Washburn, Kirkpatrick & Volkman 1996; Bidochka & Hajek 1998; Reeson *et al*. 1998).

Thus, both haemocytes and phenoloxidase play key roles in insect immunity and we can expect individuals

with high haemocyte counts and phenoloxidase activity to be better able to defend themselves against invading parasites and pathogens (e.g. Eslin & Prévost 1996, 1998; Nigam *et al*. 1997; Reeson *et al*. 1998; Kraaijeveld *et al*. 2001). Investment in prophylactic resistance mechanisms like these has been likened to investment in standing armies: the larger the army, the more readily it will be able to respond should an attack occur (e.g. Wilson 2001). In some species, THC and/or PO activity are up-regulated in response to an immune challenge (but see, e.g. Nappi, Christensen & Tracy 1987; Gillespie *et al*. 2000). However, pre- and post-challenge levels of these parameters are generally positively correlated (e.g. Eslin & Prévost 1998), indicating that basal levels provide a reliable indication of the relative threat facing the pathogen.

Larvae were reared until early in the final instar, when they were weighed and haemolymph extracted from the base of one of their rear prolegs. Just two larvae showed signs of disease, and these were excluded from the experiment. Once haemolymph had been collected, total haemocyte count and phenoloxidase activity were measured.

Total haemocyte count (THC): 15 μL of haemolymph was added to both counting chambers of a haemocytometer, and the number of haemocytes counted at a magnification of × 400 under phase contrast illumination. Each chamber was counted once and the average taken to give a single estimate for each larva.

Phenoloxidase activity (PO): 8 μL of haemolymph was added to 400 μL of ice-cold phosphate-buffered saline and mixed in a plastic Eppendorf tube. The sample was frozen at –20 °C to disrupt haemocyte membranes and PO activity in the defrosted sample was assayed spectrophotometrically using L-Dopa as a substrate (Ashida & Söderhall 1984). This involved adding 100 μL of 10 mM L-Dopa to the buffered haemolymph and pipetting triplicate samples into a 96 well microtitre plate. The absorbance was then read at 492 nm, the plate was incubated for 20 min at 25 °C and the absorbance measured once more. Subtracting the first reading from the second gives a measure of enzyme activity during the linear phase of the reaction. Using 10 μL of the haemolymph/PBS mixture, the amount of protein in the sample was also assayed using a Bio-Rad protein assay kit (calibrated using a standard curve created on the same microtitre plate using a BSA standard). Phenoloxidase activity is expressed as PO units per mg protein, where one unit is the amount of enzyme required to increase the absorbance by 0·001 per minute (note that similar results are obtained for absolute PO activity levels).

RELATIONSHIP BETWEEN IMMUNE PARAMETERS AND ENCAPSULATION RESPONSE

Although the role of haemocytes and phenoloxidase in insect immune defence is relatively well established, haemocytes in particular also have physiological functions that are not related to immune defence, such as metabolic transport, enzyme synthesis and wound healing (Chapman 1998). In order to test whether our measures do indeed reflect immune investment in Lepidoptera, we examined the correlation between THC (and PO) and the magnitude of the cellular encapsulation response. During cellular encapsulation, the invading organism is surrounded by multiple layers of haemocytes, which may then become melanized (under the action of phenoloxidase), leading to the death of the invader. Thus, as in previous studies (König & Schmid-Hempel 1995; Schmid-Hempel & Schmid-Hempel 1998; Siva-Jothy, Tsubaki & Hooper 1998; Rantala *et al*. 2000; Ryder & Siva-Jothy 2000), we assayed the encapsulation response by measuring the relative area of cells surrounding an encapsulated novel antigen (a small piece of nylon inserted into the haemocoel).

This experiment used six species of Lepidoptera: *Spodoptera exempta*, *S. littoralis* (Boisduval 1833), *Aglais urticae, Polygonia c-album, Ianachis io*, and *Vanessa atalanta*. Larvae were reared as in the earlier experiment but, following collection of haemolymph for THC and PO assays, a small length of nylon monofilament (approximately 3 mm long) was inserted into the haemocoel. Twenty-four hours later, the nylon was carefully dissected out of the larva and stored in 70% ethanol. The pieces of nylon were subsequently rehydrated, mounted on glass slides and photographed using a Polaroid digital camera. The encapsulation response was quantified by determining capsule area (the area of cells covering the nylon implant) using *Image Pro-Plus* image-analysis software. This value was then corrected for the length of nylon implant by using the residuals from the linear regression of capsule area against implant length.

A MODEL TO INVESTIGATE THE RELATIONSHIP BETWEEN HOST CLUSTERING AND EXPOSURE TO DISEASE

In order to determine the application of the results of the Watve & Jog model to ecological systems, we developed a dynamic, susceptible/infected spatially explicit model in which different degrees of host-clustering were created by allowing different proportions of local (nearest-neighbour) and distant (random) reproduction. Building on the model of Boots & Sasaki (2000), we considered a regular network of sites, each of which corresponded to either an individual host or an empty site. There were three possible states to each site: empty (0), occupied by a susceptible host (S) or occupied by an infected host (I). Infection occurs from the contact of infected and susceptible individuals at neighbouring sites at rate β. Hosts do not move between sites and infected individuals have a higher death rate ($\alpha + b$) than susceptibles (b). A site becomes empty when an individual dies, and is then available to be re-occupied

by the progeny of other individuals. The analysis is through simulation on a regular lattice with a periodic boundary, so that each site has four nearest neighbours. Host individuals reproduce at rate r into neighbouring empty sites at a proportion determined by a clustering coefficient, Q, and at random across the whole lattice with a probability $1 - Q$. Q is therefore a measure of how mixed the population is, with $Q = 1$ representing the case where reproduction occurs completely locally while when $Q = 0$ reproduction occurs at random to any site within the population. Changes in this clustering coefficient therefore produce populations with different average clustering. We counted individual infection risk through time in both endemic scenarios (where the disease is maintained in the population) and epidemic scenarios (where the disease initially spreads through the population and is then lost).

Results

INTER-SPECIFIC COMPARISON OF IMMUNE PARAMETERS

Total haemocyte count (THC) varied considerably both within and between species (Table 1). Geometric mean THC ranged between 990 (*Aglais urticae*) and 15 310 (*Spodoptera frugiperda*) haemocytes/μL; this represents more than a 15-fold difference. Across species-pairs, solitary species had consistently higher THC than the gregarious species (Fig. 1a), with a mean difference between species (within species-pairs) of 2058 ± 465 haemocytes/μL (SEM, $n = 6$), representing a 17–53% difference (38·3 ± 6·5%). The mean difference in $\log_{10}$THC between the solitary and gregarious species was significantly different from zero (two-tailed, paired t-test: $t = 4{\cdot}93$, d.f. = 5, $P = 0{\cdot}004$), and a linear mixed-effects model (with species as a fixed effect, and species-pair as a random effect) also indicated a highly significant difference between species within species-pairs ($F_{1,5} = 23{\cdot}32$, $P = 0{\cdot}005$). In other words, within matched pairs of species, the solitary species consistently had significantly higher haemocyte counts than the gregarious ones.

Phenoloxidase activity (PO) also showed marked variation within and between species (Table 1). Geometric mean PO levels ranged between 2·39 (*Aglais urticae*) and 26·79 (*Pieris brassicae*) PO units per mg protein; this represents an 11-fold difference. In five out of six species-pair comparisons, PO levels in the solitary species was higher than that in the gregarious species; in the sixth comparison (*P. napi* vs. *P. brassicae*) the difference was significant in the opposite direction (Fig. 1b). The mean difference in $\log_{10}$PO between the solitary and gregarious species was not significantly different from zero ($t = 0{\cdot}9009$, d.f. = 5, $P = 0{\cdot}41$), and $\log_{10}$PO did not differ significantly between species, within species-pairs ($F_{1,5} = 0{\cdot}39$, $P = 0{\cdot}56$). The lack of a significant difference between solitary and gregarious species is largely accounted for by the Pieridae species-pair (Fig. 1b). When this pair was excluded from the analysis, the mean difference in $\log_{10}$PO between the solitary and gregarious species became significantly different from zero ($t = 3{\cdot}54$, d.f. = 4, $P = 0{\cdot}024$), and the linear mixed-effects model yielded a significant difference between species, within species-pairs ($F_{1,4} = 12{\cdot}88$, $P = 0{\cdot}023$). Thus, in most cases we find that PO activity is higher in solitary species than gregarious ones, although the picture is more equivocal than for the THC data.

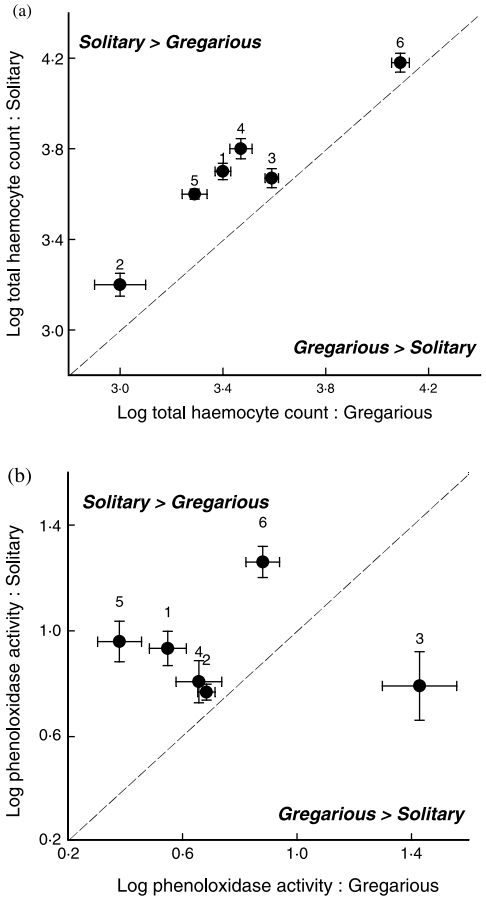

Fig. 1. Comparison of (a) $\log_{10}$ total haemocyte count and (b) $\log_{10}$ phenoloxidase activity in solitary and gregarious species of Lepidoptera. Each point represents the mean (± standard error) for a species-pair. The dashed diagonal line represents the point at which the means for the solitary and gregarious species are equal. Points above the line indicate that the values for solitary species are greater than those for the gregarious species of the pair; points below the line represent the converse. The numbers above the error bars indicate the species pair comparison (Table 1).

Body weight also varied within and between species (Table 1). However, there was no consistent variation associated with larval lifestyle; in three species-pairs, the solitary species was the heavier and, in the remaining three, the gregarious species was the heavier. The mean difference in weight between the solitary and gregarious species was not significantly different from zero ($t = 0{\cdot}621$, d.f. = 5, $P = 0{\cdot}56$), and $\log_{10}$weight did not differ significantly between species, within

species-pairs ($F_{1,5} = 0{\cdot}27$, $P = 0{\cdot}62$). Across species, there was no correlation between body weight and either $\log_{10}$THC ($r = 0{\cdot}145$, d.f. = 10, $P = 0{\cdot}65$) or $\log_{10}$PO ($r = -0{\cdot}100$, d.f. = 10, $P = 0{\cdot}76$). Moreover, after controlling for body weight in the linear mixed-effects model (by including weight as a covariate), the difference between solitary and gregarious species remained intact for $\log_{10}$THC ($F_{1,4} = 21{\cdot}85$, $P = 0{\cdot}009$) and remained non-significant for $\log_{10}$PO ($F_{1,4} = 0{\cdot}13$, $P = 0{\cdot}740$), even after excluding species-pair 3 ($F_{1,4} = 0{\cdot}31$, $P = 0{\cdot}604$). Thus, the observed trends were not a consequence of between-species differences in body weight.

IMMUNE PARAMETERS AND ENCAPSULATION RESPONSE

Across species, there was a strong positive correlation between $\log_{10}$THC and area of encapsulation ($r = 0{\cdot}977$, d.f. = 4, $P = 0{\cdot}0008$; Fig. 2a). The correlation between $\log_{10}$PO and area of encapsulation was weaker but also positive and statistically significant ($r = 0{\cdot}852$, d.f. = 4, $P = 0{\cdot}031$; Fig. 2b). Across species, $\log_{10}$THC and $\log_{10}$PO were strongly positively correlated, both within this sample of species ($r = 0{\cdot}888$, d.f. = 4, $P = 0{\cdot}018$) and overall ($r = 0{\cdot}668$, d.f. = 16, $P = 0{\cdot}0025$) (see also Fig. 3). Only the partial correlation between $\log_{10}$THC and area of encapsulation was statistically significant ($r_{\text{partial}} = 0{\cdot}916$, d.f. = 4, $P = 0{\cdot}029$; see http://faculty.vassar.edu/lowry/VassarStats.html). This indicates that, after controlling for the significant effect of total haemocyte count, PO activity level had no significant impact on the magnitude of the cellular encapsulation response ($r_{\text{partial}} = -0{\cdot}167$, d.f. = 4, $P = 0{\cdot}791$).

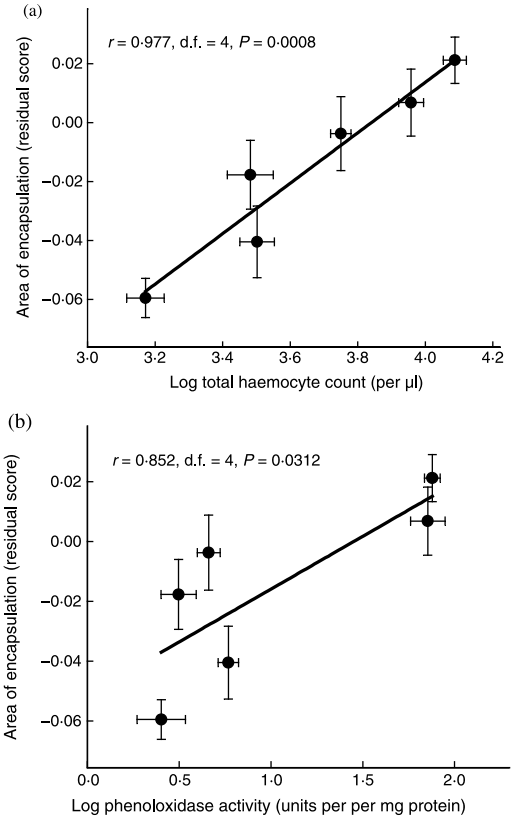

Fig. 2. Relationship between encapsulation response and (a) $\log_{10}$ total haemocyte count and (b) $\log_{10}$ phenoloxidase activity in six species of Lepidoptera. Each point represents the means (± standard error) for a single species. The THC and PO estimates were determined from haemolymph samples collected immediately prior to the insertion of the nylon implant. Area of encapsulation measures the area of cells adhering to the implant after controlling for variation in implant length (see main text).

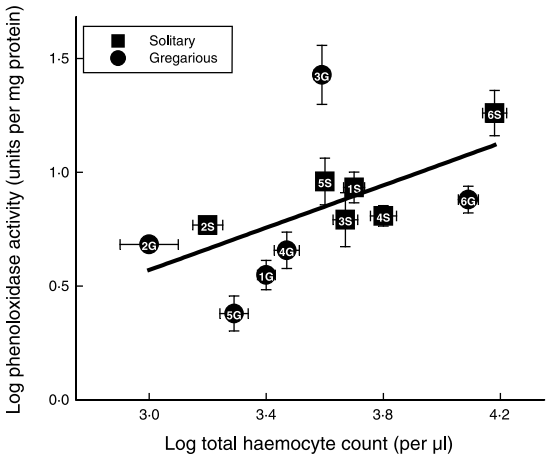

Fig. 3. Relationship between $\log_{10}$ total haemocyte count and $\log_{10}$ phenoloxidase activity in 12 species of Lepidoptera. Each point represents the means (± standard error) for a single species. Solitary-living species are indicated by the solid squares and gregariously living species by solid circles. The numbers in the symbols refer to the species-pairs indicated in Table 1.

MODEL OUTPUT

Numerous simulations consistently showed that clustering can reduce individual infection risk in both endemic host–pathogen interactions (Fig. 4a) and in epidemics (Fig. 4b,c). For example, in the epidemic case, as host aggregation increases, so the mean *per capita* infection risk decreases (Fig. 4b) and the epidemics become shorter (Fig. 4c). In general then, in a dynamical model with intrinsically generated clustering, there are many situations in which there is a lower risk of infection for individuals in more clustered populations.

Discussion

Contrary to our initial expectation, we found that total haemocyte count was significantly higher in solitary species of Lepidoptera than in gregarious ones, with THC being, on average, 40% higher in solitary-living species. In addition, phenoloxidase activity was also higher in the solitary species for five out of the six species-pairs examined. In the sixth species-pair comparison, the trend was in the opposite direction, due to

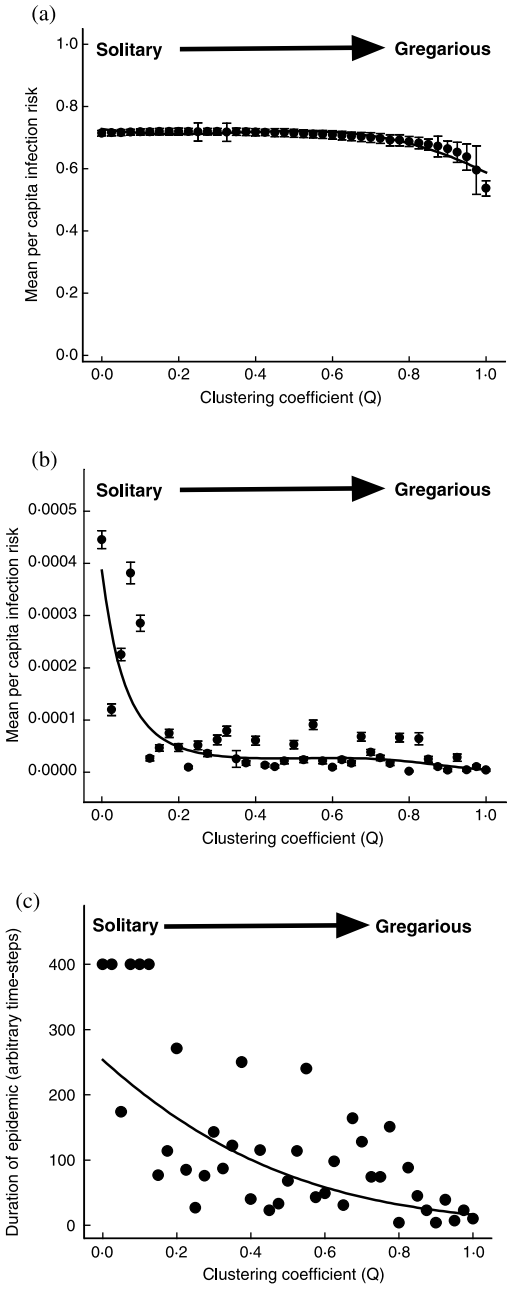

Fig. 4. Relationship between the degree of host clustering and (a) mean per capita infection risk for an endemic scenario, (b) mean per capita infection risk for an epidemic scenario, and (c) duration of an epidemic. The degree of host clustering is determined by the parameter Q (see main text). The figure shows representative output from runs of the model. The lines show the best fit exponential function; in (a) and (b) the bars represent the standard errors associated with the mean infection risk per indiviual.

the *P. brassicae* larvae exhibiting greater phenoloxidase activity than was predicted by their haemocyte counts (Fig. 3). This suggests a possible problem with the PO assays conducted on this species. Alternatively, there may be an, as yet unidentified, adaptive explanation for this contrary result. Taken together, the results from the THC and PO assays strongly suggest that there is a consistent difference between solitary and gregarious species of Lepidoptera in their levels of investment in immune function. Moreover, because of our experimental design, we can be reasonably sure that the trends are not a consequence of taxonomic artefacts (Harvey & Pagel 1991), host–parasite coevolution (Lively & Dybdahl 2000), or artefacts introduced as a result of differences in host diet (Watanabe 1987).

This result at first seems not only contrary to previously published studies (Hochberg 1991 and Møller *et al*. 2001) but also counter-intuitive. Why should gregarious species invest less in immune function than solitary ones? The answer seems to be that the expectation that gregarious species will be exposed to a higher risk of parasitism or disease is not necessarily true. The results from our model, which are in broad agreement with those generated by the epidemic model of Watve & Jog (1997), indicate that, contrary to popular belief, animals living in groups may in fact be less at risk from contagious, directly transmitted pathogens than those living solitarily. The mechanism behind this is clear: if pathogen transmission requires close proximity between potential hosts, then any process that increases the distance between infected and susceptible hosts will lead to reduced pathogen transmission. By increasing the variance in nearest-neighbour distance, host clustering increases the probability that the pathogen will fail to breach the gap between the host it is infecting and the nearest susceptible hosts. Our model indicates that part of the advantage of group-living is attributable to the fact that any disease epidemics will tend to fade out faster within populations of group-living animals than within populations of solitary hosts. Of course, group-living will fail to be advantageous in this context when the parasite or pathogen is highly mobile (or transmitted by a mobile vector) and so not constrained by the spatial distribution of its host, or when hosts are at very low densities and infection risk is low for all hosts.

The conclusions of the present study are based on the assumption that the parameters we have measured provide a good estimate of immune investment. In the case of THC there is good evidence that this is the case. For example, in the scorpionfly *Panorpa vulgaris* there was a strong positive correlation between THC and the capacity to phagocytose an artificial pathogen (silica beads) (Kürtz *et al*. 2000). Moreover, several studies (including the present one) have shown that haemocyte density is a good predictor of the magnitude of the encapsulation response stimulated by both artificial parasites (nylon monofilament) and at least some natural parasites (Eslin & Prévost 1996, 1998; Rantala *et al*. 2000; Kraaijeveld *et al*. 2001). This is important because the probability of a parasite surviving encapsulation is generally negatively correlated with the integrity of the cellular capsule surrounding it (van den Bosch 1964). There is good evidence also that phenoloxidase activity correlates well with disease resistance. Both within (Cotter & Wilson 2002) and among (Fig. 3b) Lepidopteran species, PO activity covaries with the magnitude of the encapsulation response

directed against artificial parasites. Moreover, across three phenotypes of *S. exempta* larvae, there was a positive correlation between PO activity and baculovirus LD50 (Reeson *et al*. 1998; Wilson *et al*. 2001). In tsetse flies (*Glossina* spp.), PO activity was significantly higher in a strain of *G. palpalis palpalis* that was refractory to the protozoan *Trypanosoma brucei rhodesiense* than in a strain of *G. morsitans morsitans* that was susceptible to infection (Nigam *et al*. 1997). More significantly perhaps, in *G. m. morsitans*, PO activity was significantly higher in females (the more resistant sex) than in males (the more susceptible) (Nigam *et al*. 1997). All of these examples suggest that basal levels of both THC and PO activity provide reliable indicators of the capacity of an insect to invoke haemocyte-mediated immune responses. There are exceptions, of course, to the general rule that high THC and PO equates to high resistance. For example, *D. melanogaster* lines selected for resistance to the braconid parasitoid *Asobara tabida* had higher THC than control lines, yet exhibited no significant change in resistance to the parasitoid *Leptopilina boulardi* (Fellowes, Kraaijeveld & Godfray 1999). In this case, the discrepancy is due to the fact that *L. boulardi* produces proteinaceous virus-like particles that disrupt normal haemocyte function (Rizki & Rizki 1990).

Implicit in our arguments regarding the evolution of differences in immune investment is the assumption that gregarious species of Lepidoptera find it too costly to maintain the high haemocyte counts typical of their solitary relatives (Read & Allen 2000). Whilst it is impossible at this stage to say whether or not this is true, recent studies on fruit flies suggest that haemocyte production may well be costly. In the genetic lines of *D. melanogaster* selected for resistance to *A. tabida*, increased resistance was associated with *increased* total haemocyte count (Kraaijeveld *et al*. 2001) and *decreased* feeding rate and competitive ability under conditions of food stress (Kraaijeveld & Godfray 1997; Fellowes *et al*. 1999). Moreover, across *Drosophila* species, the ability to encapsulate *A. tabida* eggs was positively correlated with THC and negatively correlated with adult metabolic rate (Eslin & Prévost 1996, 1998; Fellowes & Godfray 2000). Both of these results suggest that haemocyte production is costly and may be traded-off against metabolic and feeding rate (Fellowes & Godfray 2000).

We have argued that differences in immune function between solitary and gregariously feeding species reflect their relative risk of infection. However, an alternative explanation for our results is that the infection risks for solitary and gregarious species are comparable, but the gregarious species have fewer resources available to invest in immune defence because of their greater investment in other functions, such as somatic growth or competitive ability. In the selection experiments described above, Kraaijeveld *et al*. (2001) found that in there was a negative genetic correlation between larval competitive ability and immune defence. Thus, it is possible that the gregarious species invest relatively more in competitive ability than solitary species, at the expense of their immune systems, leading to an association between larval feeding habit and immune function. However, since aggregations of Lepidopteran larvae tend to comprise related individuals, kin selection is likely to lead to the evolution of *reduced* rather than enhanced competitive ability in gregarious species (Hamilton 1964; West, Pen & Griffin 2002). We therefore think that it is unlikely that this mechanism explains our results.

Another possibility is that the solitary and gregarious species we used in our experiments responded differently to larval density. Recent studies on Lepidoptera, and other insect taxa, indicate that some insect species exhibit density-dependent prophylactic investment in immune function (e.g. Reeson *et al*. 1998; Wilson & Reeson 1998; Barnes & Siva-Jothy 2000; Wilson *et al*. 2001), such that pathogen resistance is enhanced under crowded conditions, as an adaptation to the greater threat from pathogens at higher population densities (Wilson & Reeson 1998). Thus, it is possible that although all species were reared at the same larval density prior to sampling, the solitary species exhibited a greater prophylactic response to being crowded than the gregarious species. Whilst we cannot fully discount this explanation, it is noteworthy that the magnitude of the observed difference in THC and PO between sister-species (Table 1) is much greater than any observed density-dependent increase in immune function (e.g. Reeson *et al*. 1998; Barnes & Siva-Jothy 2000).

We have argued here that solitary-living species invest more in immune function as an adaptation to counter the increased risk from directly transmitted, contagious parasites and pathogens, such as bacteria and viruses. However, THC and PO are also both involved in the encapsulation of parasitoid eggs (e.g. Eslin & Prévost 1996, 1998; Gillespie *et al*. 1997; Kraaijeveld *et al*. 2001), and therefore an alternative hypothesis is that the difference in immune function has evolved because solitary caterpillars are more likely to be attacked by parasitoids than gregarious ones. Sheehan (1991) analysed a very large parasitoid dataset from North America and found that gregarious Lepidoptera tended to suffer higher levels of parasitism from generalist parasitoids than solitary species. However, a re-analysis of this dataset controlling for host phylogeny and sampling effort found no consistent relationship between lifestyle (gregarious vs. solitary) and parasitism rate (R. Knell & K. Wilson, unpublished), suggesting that the differential investment in immune function observed in solitary and gregarious caterpillars is not a consequence of their relative rates of parasitoid attack. Clearly, the best way to determine the adaptive value of the observed difference in immune function between solitary and gregarious species, is to challenge them with a range of parasites and pathogens, including generalist parasitoids. Whilst these types of experiments are feasible, they are logistically difficult because it is important to challenge species-pairs

simultaneously in comparable conditions with pathogens to which neither species has evolved a specific defence mechanism.

If, as we have argued, our measures of immune function genuinely reflect the extent of immune investment in these insects, how can we reconcile our results with those from the studies of Hochberg (1991) and Møller *et al*. (2001)? Hochberg discussed some of the problems with his analysis, which we have mentioned above. Further to this we can add that what Hochberg found was not a difference in the overall level of investment in immune function, but a difference in age-related resistance between solitary and gregarious Lepidoptera, such that gregarious species exhibited an increase in virus resistance in the later larval instars that was not found in solitary species. This age-related increase in virus resistance could, in fact, reflect different patterns of age-related risk between solitary and gregarious larvae that are independent of the overall differences in risk. Gregarious species may increase their feeding rates in the later larval instars more than solitary species, for example, which would increase the risk of being infected with pathogens transmitted via contaminated foliage, such as baculoviruses.

There are at least two potential explanations for the conflicting results of the present study and that of Møller *et al*. (2001). First, whilst our study measured constituitive levels of components of the insect innate immune system, Møller *et al*. studied the avian acquired immune response. Immune responsiveness in the avian system may therefore reflect current levels of activation (which may be confounded by present or previous infections) rather than evolved genetic differences.

Second, the apparently contradictory conclusions of the two studies may be resolved by considering the details of the social systems in the two groups studied. In colonially nesting birds, the colonies form only during the breeding season, and throughout the remainder of the year the birds come into frequent contact with individuals from other colonies. In contrast, in gregariously feeding Lepidoptera, groups of caterpillars may never encounter each other during their susceptible larval stages. The higher contact rate between bird colonies is likely to lead to relatively higher rates of parasitism in more colonial birds, while the low rates of contact between caterpillar groups will lead to relatively low rates of parasitism in comparison to solitary animals. Taken together then, our results and those of Møller *et al*. suggest that rather than a simple relationship between sociality and parasitism it is instead likely that the specific details of the social system in question will determine whether animals experience increased or decreased risks of parasitism.

Acknowledgements

We are extremely grateful to two anonymous referees for their comments on a previous version of this manuscript. This work was funded by a grant to K.W. from the Natural Environment Research Council, UK, and was conducted whilst K.W. and M.B. were in receipt of NERC-UK Advanced Research Fellowships.

References

Alexander, R.D. (1974) The evolution of social behaviour. *Annual Review of Ecology and Systematics*, **5**, 325–383.

Anderson, R.M. & May, R.M. (1979) Population biology of infectious diseases: Part I. *Nature*, **280**, 361–367.

Ashida, M. & Söderhall, K. (1984) The prophenoloxidase activating system in crayfish. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology*, **77**, 21–26.

Barnes, A.I. & Siva-Jothy, M.T. (2000) Density-dependent prophylaxis in the mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **267**, 177–182.

Bidochka, M.J. & Hajek, A.E. (1998) A nonpermissive entomophthoralean fungal infection increases activation of insect prophenoloxidase. *Journal of Invertebrate Pathology*, **72**, 231–238.

Boots, M. & Sasaki, A. (2000) The evolutionary dynamics of local infection and global reproduction in host–parasite interactions. *Ecology Letters*, **3**, 181–185.

van den Bosch, R. (1964) Encapsulation of the eggs of *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) in larvae of *Hypera brunneipennis* (Boheman) and *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). *Journal of Insect Pathology*, **6**, 343–367.

Chapman, R.F. (1998) *The Insects: Structure and Function*, 4th edn. Cambridge University Press, Cambridge.

Coté, I.M. & Poulin, R. (1995) Parasitism and group-size in social animals – a metaanalysis. *Behavioral Ecology*, **6**, 159–165.

Cotter, S.C. & Wilson, K. (2002) Heritability of immune function in the caterpilar, *Spodoptera littoralis*. *Heredity*, **88**, 229–234.

Davies, C.R., Ayres, J.M., Dye, C. & Deane, L.M. (1991) Malaria infection-rate of Amazonian primates increases with body-weight and group-size. *Functional Ecology*, **5**, 655–662.

Dobson, A.P. (1988) The population biology of parasite-induced changes in host behavior. *Quarterly Review of Biology*, **63**, 139–165.

Eslin, P. & Prévost, G. (1996) Variation in *Drosophila* concentration of haemocytes associated with different ability to encapsulate *Asobara tabida* larval parasitoid. *Journal of Insect Physiology*, **42**, 549–555.

Eslin, P. & Prévost, G. (1998) Hemocyte load and immune resistance to *Asobara tabida* are correlated in species of the *Drosophila melanogaster* subgroup. *Journal of Insect Physiology*, **44**, 807–816.

Fellowes, M.D.E. & Godfray, H.C.J. (2000) The evolutionary ecology of resistance to parasitoids by *Drosophila*. *Heredity*, **84**, 1–8.

Fellowes, M.D.E., Kraaijeveld, A.R. & Godfray, H.C.J. (1999) Association between feeding rate and parasitoid resistance in *Drosophila melanogaster*. *Evolution*, **53**, 1302–1305.

Freeland, W.J. (1979) Primate social groups as biological islands. *Ecology*, **60**, 719–728.

Gillespie, J.P., Burnett, C. & Charnley, A.K. (2000) The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus,

Metarhizium anisopliae var. *acridum*. *Journal of Insect Physiology*, **46**, 429–437.

Gillespie, J.P., Kanost, M.R. & Trenczek, T. (1997) Biological mediators of insect immunity. *Annual Review of Entomology*, **42**, 611–643.

Gomez, S.A., Moscardi, F. & Sosa-Gomez, D.R. (1999) *Spodoptera frugiperda* susceptibility to nuclear polyhedrosis virus geographical isolates. *Pesquisa Agropecuaria Brasileira*, **34**, 1539–1544.

Gupta, A.P. (ed.) (1991) *Immunology of Insects and Other Arthropods*. CRC Press, Boca Raton, Florida.

Hagen, H.E., Grunewald, J. & Hamm, P.J. (1994) Induction of the prophenoloxidase-activating system of *Simulium* (Diptera: Simuliidae) following *Onchocerca* (Nematoda: Filaroidea) infection. *Parasitology*, **109**, 649–655.

Hamilton, W.D. (1964) The genetical theory of social evolution. I and II. *Journal of Theoretical Biology*, **6**, 1–52.

Harvey, P.H. & Pagel, M. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Hochberg, M.E. (1991) Viruses as costs to gregarious feeding behavior in the Lepidoptera. *Oikos*, **61**, 291–296.

Hung, S.Y. & Boucias, D.G. (1996) Phenoloxidase activity in hemolymph of naive and *Beauveria bassiana*-infected *Spodoptera exigua* larvae. *Journal of Invertebrate Pathology*, **67**, 35–40.

König, C. & Schmid-Hempel, P. (1995) Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terrestris* L. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **260**, 225–227.

Kraaijeveld, A.R. & Godfray, H.C.J. (1997) Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature*, **389**, 278–280.

Kraaijeveld, A.R., Limentani, E.C. & Godfray, H.C.J. (2001) Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **268**, 259–261.

Kürtz, J., Wiesner, A., Götz, P. & Sauer, K.P. (2000) Gender differences and individual variation in the immune system of the scorpionfly *Panorpa vulgaris* (Insecta: Mecoptera). *Developmental and Comparative Immunology*, **24**, 1–12.

Lively, C.M. & Dybdahl, M.F. (2000) Parasite adaptation to locally common host genotypes. *Nature*, **405**, 679–681.

McCallum, H., Barlow, N. & Hone, J. (2001) How should pathogen transmission be modelled? *Trends in Ecology and Evolution*, **16**, 295–300.

Møller, A.P., Merino, S., Brown, C.R. & Robertson, R.J. (2001) Immune defense and host sociality: a comparative study of swallows and martins. *American Naturalist*, **158**, 136–145.

Nappi, A.J., Christensen, B.M. & Tracy, J.W. (1987) Quantitative analysis of hemolymph monophenol oxidase activity in immune reactive *Aedes aegypti*. *Insect Biochemistry*, **17**, 685–688.

Nigam, Y., Maudlin, I., Welburn, S. & Ratcliffe, N.A. (1997) Detection of phenoloxidase activity in the hemolymph of tsetse flies, refractory and susceptible to infection with *Trypanosoma brucei rhodesiense*. *Journal of Invertebrate Pathology*, **69**, 279–281.

Nunn, C.L., Gittleman, J.L. & Antonovics, J. (2000) Promiscuity and the primate immune system. *Science*, **290**, 1168–1170.

Ourth, D.D. & Renis, H.E. (1993) Antiviral melanisation reaction of *Heliothis virescens* haemolymph against DNA and RNA viruses *in vitro*. *Comparative Biochemistry and Physiology*, **105B**, 719–723.

Pech, L.L. & Strand, M.R. (1995) Encapsulation of foreign targets by hemocytes of the moth *Pseudoplusia includens* (Lepidoptera, Noctuidae) involves an Rgd-dependent cell-adhesion mechanism. *Journal of Insect Physiology*, **41**, 481–488.

Poiani, A. (1992) Ectoparasitism as a possible cost of social life – a comparative-analysis using Australian passerines (Passeriformes). *Oecologia*, **92**, 429–441.

Poulin, R. (1991a) Group-living and infestation by ectoparasites in passerines. *Condor*, **93**, 418–423.

Poulin, R. (1991b) Group-living and the richness of the parasite fauna in Canadian freshwater fishes. *Oecologia*, **86**, 390–394.

Ranta, E. (1992) Gregariousness versus solitude – another look at parasite faunal richness in Canadian freshwater fishes. *Oecologia*, **89**, 150–152.

Rantala, M.J., Koskimaki, J., Taskinen, J., Tynkkynen, K. & Suhonen, J. (2000) Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens* L. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **267**, 2453–2457.

Ratcliffe, N.A. (1993) Cellular defense responses of insects: unresolved problems. *Parasites and Pathogens of Insects* (eds N.E. Beckage, S.N. Thompson & B.A. Federici), Vol. 1, Parasites, pp. 267–304. Academic Press, Inc., San Diego.

Ratcliffe, N.A. & Rowley, A.F. (1987) Insect responses to parasites and other pathogens. *Immune Responses in Parasitic Infections: Immunology, Immunopathology, and Immunoprophylaxis* (eds E.J.L. Soulsby), Vol. 4, pp. 271–332. CRC Press, Boca Raton, FL.

Read, A.F. & Allen, J.E. (2000) Evolution and immunology – the economics of immunity. *Science*, **290**, 1104–1105.

Reeson, A.F., Wilson, K., Gunn, A., Hails, R.S. & Goulson, D. (1998) Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **265**, 1787–1791.

Rizki, R.M. & Rizki, T.M. (1990) Parasitoid virus-like particles destroy *Drosophila* cellular immunity. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 8388–8392.

Rowley, A.F., Brookman, J.L. & Ratcliffe, N.A. (1990) Possible Involvement of the prophenoloxidase system of the locust, *Locusta migratoria*, in antimicrobial activity. *Journal of Invertebrate Pathology*, **56**, 31–38.

Ryder, J.J. & Siva-Jothy, M.T. (2000) Male calling song provides a reliable signal of immune function in a cricket. *Proceedings of the Royal Society of London Series B – Biological Sciences*, **267**, 1171–1175.

Schmid-Hempel, R. & Schmid-Hempel, P. (1998) Colony performance and immunocompetence of a social insect, *Bombus terrestris*, in poor and variable environments. *Functional Ecology*, **12**, 22–30.

Semple, S., Cowlinshaw, G. & Bennett, P. (2002) Immune system evolution among anthropoid primates: parasites, injuries and predators. *Proceedings of the Royal Society of London Series B –Biological Sciences*, **269**, 1031–1037.

Sheehan, W. (1991) Host range patterns of hymenopteran parasitoids of exophytic Lepidopteran folivores. *Insect–Plant Interactions* (ed. E. Bernays), Vol. III, pp. 209–248. CRC Press, Boca Raton, Fl.

Siva-Jothy, M.T., Tsubaki, Y. & Hooper, R.E. (1998) Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. *Physiological Entomology*, **23**, 274–277.

Tanada, Y. & Fuxa, J. (1987) The pathogen population. *Epizootiology of Insect Diseases* (eds J. Fuxa & Y. Tanada), pp. 113–157. John Wiley & Sons, New York.

Washburn, J.O., Kirkpatrick, B.A. & Volkman, L.E. (1996) Insect protection against viruses. *Nature*, **383**, 767–767.

Watanabe, H. (1987) The host population. *Epizootiology of Insect Diseases* (eds J. Fuxa & Y. Tanada), pp. 71–112. John Wiley & Sons, New York.

Watve, M.G. & Jog, M.M. (1997) Epidemic diseases and host

clustering: an optimum cluster size ensures maximum survival. *Journal of Theoretical Biology*, **184**, 167–171.

Watve, M.G. & Sukumar, R. (1995) Parasite abundance and diversity in mammals – correlates with host ecology. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 8945–8949.

West, S.A., Pen, I. & Griffin, A.S. (2002) Conflict and cooperation – cooperation and competition between relatives. *Science*, **296**, 72–75.

Wilson, K. (2001) The costs of resistance in *Drosophila*: blood cells count. *Trends in Ecology and Evolution*, **16**, 72–73.

Wilson, K., Cotter, S.C., Reeson, A.F. & Pell, J.K. (2001) Melanism and disease resistance in insects. *Ecology Letters*, **4**, 637–649.

Wilson, K. & Reeson, A.F. (1998) Density-dependent prophylaxis: evidence from Lepidoptera–baculovirus interactions? *Ecological Entomology*, **23**, 100–101.

Received 10 June 2002; accepted 18 September 2002