

Immune defence, dispersal and local adaptation

Joachim Kurtz,* Kirsten Klappert, Wolfgang Schneider and Klaus Reinhold

*Institute for Evolution and Ecology, University of Bonn, An der Immenburg 1,
D-53121 Bonn, Germany*

ABSTRACT

To determine the influence of dispersal on the expression of immune traits, we conducted a reciprocal transfer experiment. *Chorthippus biguttulus* grasshoppers from two populations were released as juveniles into their native and transfer environments. After recapture as adults, we found that an immune trait, the amount of phagocytically active cells, was significantly reduced in the transfer environments. In contrast, adult body mass differed between the two habitats, but was not reduced in the transfer environments. The results suggest that dispersal to a new environment can reduce the expression of immune traits, while otherwise not influencing body condition. One reason for such an effect could be that the parasite community in the foreign environment might be relatively maladapted, which would lead to reduced demands for resource allocation to immune traits.

Keywords: *Chorthippus biguttulus*, dispersal, host resistance, immunocompetence, invertebrate immunity, local adaptation, phagocytosis, reciprocal transfer.

INTRODUCTION

It is increasingly believed that the expression of immune traits involves costs (Kraaijeveld and Godfray, 1997; Møller *et al.*, 1998; Coustau *et al.*, 2000; Lochmiller and Deerenberg, 2000; Moret and Schmid-Hempel, 2000; Råberg *et al.*, 2000). Therefore, immunity should be determined by the availability of resources necessary for immune function and by the demand for immune defence (Sheldon and Verhulst, 1996). These factors are often largely influenced by the environment. Environmental conditions might influence the availability of resources limiting immune function (Bortolotti *et al.*, 1996; Grether *et al.*, 1999). Furthermore, the impact of parasites could differ between environments. As a consequence, immune functions might vary between environments (Møller, 1998). We therefore expect that immune defence varies depending on whether individuals have dispersed or whether they are living in the parental location.

Animals are often adapted to diverse local parameters, such as climate, habitat structure, food properties or competitors (Wade, 1990; DeMeester, 1996). In particular, parasites often (but not always) perform better on local than on foreign hosts (Lively, 1989; Ebert,

* Address all correspondence to Joachim Kurtz, Max Planck Institute for Limnology, Department of Evolutionary Ecology, August-Thienemann-Str. 2, D-24306 Plön, Germany. e-mail: kurtz@mpil-ploen.mpg.de
Consult the copyright statement on the inside front cover for non-commercial copying policies.

1994; Gandon *et al.*, 1996; Lively and Jokela, 1996; Morand *et al.*, 1996; Oppliger *et al.*, 1999; Lively and Dybdahl, 2000; Kraaijeveld and Godfray, 2001).

The effect of dispersal on the expression of immune traits will depend on both local adaptation of the dispersed animals themselves and local adaptation of relevant parasites. Dispersers will encounter a new environment they might not optimally be adapted to, which possibly results in reduced body condition and, as a consequence, decreased immunocompetence. On the other hand, parasites encountered in the foreign environment might not be optimally adapted to dispersers, reducing the demand for immune defence. In this case, it could theoretically be advantageous for animals to disperse, thereby escaping from a locally adapted parasite community.

Artificial transfer of individuals to a foreign environment could be a means of testing the effect of dispersal on immune function. We transferred *Chorthippus biguttulus* grasshoppers reciprocally between two populations and compared phagocytic capacity (as an estimate of general immunocompetence) after a growth period in the native and transfer environments. In addition, we compared body mass to determine whether effects on immunity are a consequence of differences in general body condition.

METHODS

The study species

The nightingale grasshopper *Chorthippus biguttulus*, Ramme (Caelifera, Acrididae, Gomphocerinae) is one of the most common local grasshoppers in Germany. This species produces one generation per year with hibernating eggs. Larvae emerge in May and moult four to five times until adulthood is reached around July or August. All stadiums live at moderate dry spots and feed on diverse species of grasses. As far as we know, there are no data on dispersal in *Ch. biguttulus*, but there is evidence for considerable dispersal in other *Chorthippus* species (Bridle *et al.*, 2001). Parasites have also been studied in other *Chorthippus* species, where gregarines seem to be most prominent (Reinhardt, 2001).

The reciprocal transfer experiment

We transferred grasshoppers between two populations, 'Siegaue' (7°10' E, 50°46' N, altitude 45 m, a meadow on the south side of a dike) and 'Eifel' (7°02' E, 50°23' N, altitude 600 m, a steep and dry southward slope of the Eifel mountains), separated by a distance of about 40 km. To avoid prior contact with parasites of the source habitat or simultaneous transfer of parasites already present in the hosts, we used laboratory-bred offspring of grasshoppers captured in the field.

In August 1998, we caught about 120 females and 60 males from each population and held them in the laboratory. The eggs laid by these females were stored from November to April at 4°C to simulate hibernation. Subsequently, we transferred the eggs to room temperature to induce hatching. About 1 day after hatching, we marked all larvae according to their parents' origin by cutting either the left or right antenna. We assume that the grasshoppers are symmetrical and that mutilation of the left or right antenna has a similar effect. Since we only compared the two types of mutilated grasshoppers, potential effects of mutilation on immunocompetence should be similar in both groups. Within 2 days of marking, the grasshopper hatchlings were released to both field populations. Altogether,

2016 and 1955 marked larvae from the Eifel were transferred to the native and the foreign habitats, respectively. Similarly, 1887 and 1920 marked hatchlings from the Siegaue were released in the native and foreign habitats. In August we collected all marked individuals and recorded their body mass. Altogether, we collected 203 native individuals (84 and 119 from the Eifel and the Siegaue, respectively) and 181 transferred individuals (70 and 111 from the Eifel and the Siegaue, respectively). We were able to recapture more grasshoppers from the Siegaue ($\chi^2_1 = 17.04$, $P < 0.0001$), but recapturing rates did not differ between the source populations ($\chi^2_1 = 0.011$, $P = 0.915$) or between native and transferred grasshoppers ($\chi^2_1 = 1.108$, $P = 0.293$). We brought recaptured grasshoppers to the laboratory and collected data on mating behaviour, which was the main focus of the reciprocal transfer experiment (K. Reinhold and K. Klappert, submitted). Grasshoppers were therefore used for song-recordings and mate-choice experiments before assessing their immunocompetence. During that time, they were held in the laboratory (20°C, 18 h daylength and supplied with fresh grass *ad libitum*). Immunocompetence was assessed 18 ± 5 days (mean $\pm$ s) after recapture. For testing immunocompetence, 49 native males (23 and 26 from the Eifel and the Siegaue populations, respectively) and 48 transferred males (27 and 21 recaptured from the Eifel and the Siegaue, respectively) were randomly selected from all recaptured animals. We only used males to reduce possible variation due to differences in immune traits between males and females.

Measuring immunocompetence: *in vitro* phagocytosis

As an estimate of general immunocompetence, we measured phagocytosis of fluorescently labelled hydrophilic silica beads by grasshopper haemocytes (immune cells) in an *in vitro* quenching assay. This assay has been described previously by Kurtz and Sauer (1999) and Kurtz *et al.* (2000) for the scorpionfly *Panorpa vulgaris*. For the grasshopper haemocytes, we made the following small changes.

To facilitate haemolymph (insect blood) collection, we moistened the grasshoppers 30 min before bleeding. Furthermore, 15 min before bleeding, they were injected dorsally (between the metasternite and the first abdominal sternite) with 3 μ l of anticoagulant buffer containing 0.2 mg $\cdot$ ml⁻¹ phenylthiourea (PTU), using a 5 μ l Hamilton syringe. To obtain 2 μ l of haemolymph from each individual (on a few occasions, slightly less than 2 μ l was obtained), the left hind leg was cut and haemolymph was collected with a calibrated capillary, coated with the anticoagulant PTU the day before (by filling the capillary with 98.6% ethanol saturated with PTU and subsequent drying under sterile conditions). Haemolymph was immediately drained into 50 μ l ice-cold anticoagulant buffer (69 mmol $\cdot$ l⁻¹ KCl, 27 mmol $\cdot$ l⁻¹ NaCl, 2 mmol $\cdot$ l⁻¹ NaHCO₃, 100 mmol $\cdot$ l⁻¹ D(+)-glucose, 30 mmol $\cdot$ l⁻¹ tripotassium citrate, 26 mmol $\cdot$ l⁻¹ citric acid, 20 mmol $\cdot$ l⁻¹ Na₂-EDTA) and subsequently used to prepare cell monolayers for phagocytosis as described for *P. vulgaris*, except that samples were incubated for only 1 h at 30°C.

For analysis, we determined the percentage phagocytosis (PP, i.e. percentage of cells containing silica beads) and the phagocytic index (PI, i.e. average amount of beads taken up per active cell), counting (at 200 $\times$ magnification) five areas of 0.39 mm² each (resulting in 6% of the whole culture area), but at least 300 cells per monolayer.

The percentage phagocytosis and the phagocytic index were normally distributed (Shapiro-Wilk test: $P > 0.5$), positively correlated ($r = 0.411$, $P < 0.0001$, $n = 97$) and independent of body mass (PP: $r = 0.016$, $P = 0.880$; PI: $r = 0.128$, $P = 0.212$; $n = 97$). There

was no effect of the time individuals were held in the laboratory (PP: $r = -0.053$, $P = 0.610$; PI: $r = 0.079$, $P = 0.436$; $n = 97$). Phagocytosis was determined in batches of up to 12 individual haemolymph samples, collected on the same day and attributed to the same microscopic slide for the assay. Some of the variation in the percentage phagocytosis and the phagocytic index might, therefore, be induced by resemblance between these batches. To control for this, we included 'batch' as a random effect in the statistical models. In contrast to the analysis in *P. vulgaris*, the amount of cells in each haemolymph sample was not considered, as this value was probably influenced by the anticoagulant injection and, therefore, was not a good estimator of haemocyte load.

Statistical analyses were performed with the JMP 4.0 for Macintosh software (SAS Institute Inc.), using standard least square fitting with the expected mean square approach. We constructed linear contrasts to make the following comparisons:

1. Are the means of the transferred populations different from the means of the untransferred controls?
2. Do the two source populations differ in their home locations?
3. Do the two source populations differ in their transferred locations?

We calculated statistical power according to Cohen (1988). Statistical power is defined as the chance of obtaining a significant result, given that there is an effect. By convention, a power of 0.80 (i.e. an 80% chance) is normally considered sufficient.

RESULTS

In the transfer environments, individuals had a slightly reduced proportion of phagocytically active haemocytes than those growing up in their native environments (Fig. 1, Table 1). A similar trend was observed for the amount of ingested particles per phagocyte, but this effect was weaker and not statistically significant (Fig. 1, Table 1). Effect sizes can be estimated from our study: transfer to the foreign environment led to an $11.4 \pm 5.6\%$ reduction in phagocytically active haemocytes and a $5.8 \pm 3.7\%$ reduction in ingested particles per phagocyte. The latter value was not significantly different from zero effect. Power analysis showed that we would have needed a sample of 154 individuals to have had such an effect significant in our study. However, the power of our study was sufficient to detect a medium effect of a 20% reduction (power = 0.999) and almost sufficient to detect a small effect of 10% (power = 0.795). We can therefore conclude that, if there was an effect of transfer on ingested particles per phagocyte, it was most likely only a small effect, not exceeding 20%. For both phagocytically active haemocytes and ingested particles per phagocyte, there were no differences between the source populations, neither when reared in their home locations, nor in the transferred locations (Table 1).

Transfer to a foreign environment did not have a significant influence on body mass (Fig. 1, Table 1). The effect size of a possible reduction in body mass in the foreign environment, estimated from our study, was very low ($1.9 \pm 1.8\%$). The power of our study was sufficient to detect even a small effect of 10% (power = 0.997). The two source populations differed strongly in body mass, both when growing up in their home locations and in the transferred locations. This shows that the two rearing habitats led to significant differences in body mass, irrespective of the source population (individuals reared in the Eifel were on average 11% heavier).

Table 1. Analyses of variance for the effects of reciprocal transfer of *Chorthippus biguttulus* grasshoppers to a foreign habitat on percentage phagocytosis, phagocytic index and body mass

	SS	d.f.	<i>F</i>	<i>P</i>
Percentage phagocytosis				
Group	1451	3	2.325	0.081
Batch	4540	10	2.182	0.027
Error	17274	83		
<i>Contrasts</i>				
Transferred <i>vs</i> untransferred	827	1	3.975	0.049
S <i>vs</i> E (in home locations)	521	1	2.505	0.117
S <i>vs</i> E (in transferred locations)	380	1	1.826	0.180
Phagocytic index				
Group	1.29	3	1.199	0.315
Batch	12.69	10	3.545	<0.001
Error	29.70	83		
<i>Contrasts</i>				
Transferred <i>vs</i> untransferred	0.88	1	2.455	0.121
S <i>vs</i> E (in home locations)	0.27	1	0.744	0.391
S <i>vs</i> E (in transferred locations)	0.02	1	0.059	0.809
Body mass				
Group	1628	3	10.89	<0.001
Error	4634	93		
<i>Contrasts</i>				
Transferred <i>vs</i> untransferred	56	1	1.12	0.291
S <i>vs</i> E (in home locations)	904	1	18.14	<0.001
S <i>vs</i> E (in transferred locations)	709	1	14.23	<0.001

Note: The main effect 'group' denotes the four treatment groups – the two source populations (Siegaue = S and Eifel = E) each released into the native and transfer environments. Similarity of phagocytosis of individuals measured on the same day and on the same microscopic slide was controlled for by using 'batch' as an additional factor. Contrasts were constructed to compare: (1) whether the means of transferred populations differed from those of the untransferred populations; (2) whether the two source populations differed in their home locations; and (3) whether the two source populations differed in their transferred locations.

In summary, we conclude that body mass differed between rearing habitats, but not between transfer and native environments, while phagocytic capacity was slightly reduced in the transfer environments.

DISCUSSION

Using a reciprocal transfer experiment, we compared the immunocompetence of grasshoppers in their native and transfer environments. The grasshoppers showed a slightly reduced phagocytic capacity in the transfer environments. This effect was small but significant for the proportion of phagocytic cells, while there was only a trend for the number of particles taken up per phagocyte. Body mass upon recapture differed with respect to the

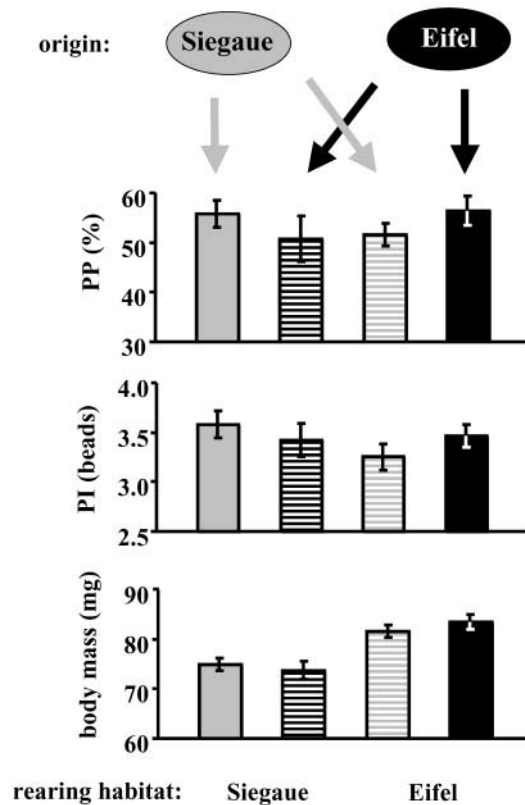


Fig. 1. Effect of reciprocal transfer between two populations on percentage phagocytosis (PP = % phagocytically active haemocytes), phagocytic index (PI = phagocytosed particles per active cell) and body mass upon recapture of *Chorthippus biguttulus* grasshoppers (mean $\pm$ standard error). Hatched bars are transferred animals; sample size was between 21 and 27 individuals per group. See Table 1 for statistical analysis.

habitat where individuals grew up, suggesting that the two habitats differed in some environmental conditions. However, there was no effect of transfer on body mass, indicating that the foreign environment did not reduce growth or body condition. It is possible to conclude, therefore, that dispersal to a new environment can reduce the expression of immune traits, while otherwise not influencing body condition.

Immune traits are increasingly being studied in evolutionary ecology (Sheldon and Verhulst, 1996; Johnsen and Zuk, 1999; Norris and Evans, 2000; Read and Allen, 2000; Siva-Jothy, 2000). For example, female mate choice based on male ornament traits signalling superior immunocompetence (Folstad and Karter, 1992) is considered to be adaptive for females in particular when offspring inherit beneficial paternal traits (Kurtz and Sauer, 1999; Ryder and Siva-Jothy, 2000, 2001; Barber *et al.*, 2001). Knowledge about the phenotypic expression of immune traits under ecologically relevant conditions is therefore needed.

Until now, dispersal has rarely been considered in ecological immunology. A possible effect of migration on immunocompetence was mainly considered in terms of long-distance

migration, for example birds migrating to the tropics. In such circumstances, a larger impact of parasites on migrants is expected, because of a generally higher parasite pressure in the tropics, exposure to parasites during migration and the endurance required during migratory flights (Piersma, 1997; Møller, 1998; Møller and Erritzoe, 1998). Recently, Altizer (2001) demonstrated higher host resistance in long-distance migratory populations of monarch butterflies, which might be explained by stronger selection against susceptible hosts in migratory populations.

Recently, Møller and Erritzoe (2001) argued that exposure to local parasite communities during natal dispersal might even serve as a means of 'vaccination' against local parasites. We used invertebrates in our study, which lack an adaptive immune system, relying instead on innate immunity (Gillespie *et al.*, 1997). Therefore, a 'vaccination' benefit of dispersal is unlikely to occur in invertebrates. But there might be another benefit of dispersal with respect to local parasite adaptation: dispersers might be confronted with a relatively maladapted parasite community. The release of parasite pressure on dispersers might not only lead to lower parasite-induced mortality, but also to a lower investment in resources for immunocompetence. If this is the case, dispersers could, for example, allocate surplus resources in ornament traits (Folstad and Karter, 1992; Sheldon and Verhulst, 1996), thereby increasing reproductive success. Females choosing dispersers might gain, if parasites are less adapted to the offspring of dispersers than those of locals.

The reduction in immunity in the absence of any effect on body mass in our study could indicate that transferred animals indeed reduced allocation to immune defence as a consequence of relatively maladapted parasites in the transfer environment, rather than because of their own decreased performance. However, comparing body mass of foreign and native individuals does not rule out the possibility that physiological traits that do not impinge on body mass are altered in transferred animals. Therefore, additional fitness parameters should be included in further studies. The relative importance of host and parasite local adaptation might depend on the current impact of parasites and it might, therefore, fluctuate over time.

To evaluate parasite pressure, detailed knowledge of relevant parasites and their local adaptations would be useful. Therefore, as a definitive test of the idea that dispersal might be beneficial when parasites are locally adapted, we suggest studying immunocompetence in populations for which more information on local adaptations of relevant parasites is available.

ACKNOWLEDGEMENTS

We thank Klaus Peter Sauer for his support and Curtis Lively for his helpful comments on an earlier draft of the manuscript. The study was funded by grant RE 1167/3-1 from the Deutsche Forschungsgemeinschaft.

REFERENCES

- Altizer, S.M. 2001. Migratory behaviour and host-parasite co-evolution in natural populations of monarch butterflies infected with a protozoan parasite. *Evol. Ecol. Res.*, **3**: 611–632.
- Barber, I., Arnott, S.A., Braithwaite, V.A., Andrew, J. and Huntingford, F.A. 2001. Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B*, **268**: 71–76.

- Bortolotti, G.R., Negro, J.J., Tella, J.L., Marchant, T.A. and Bird, D.M. 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. *Proc. R. Soc. Lond. B*, **263**: 1171–1176.
- Bridle, J.R., Baird, S.J.E. and Butlin, R.K. 2001. Spatial structure and habitat variation in a grass-hopper hybrid zone. *Evolution*, **55**: 1832–1843.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioural Sciences*. Hillsdale, NJ: Lawrence Erlbaum.
- Coustau, C., Chevillon, C. and Ffrench-Constant, R. 2000. Resistance to xenobiotics and parasites: can we count the cost? *Trends Ecol. Evol.*, **15**: 378–383.
- DeMeester, L. 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience*, **3**: 385–399.
- Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science*, **265**: 1084–1086.
- Folstad, I. and Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.*, **139**: 603–622.
- Gandon, S., Capowiez, Y., Dubois, Y., Michalakakis, Y. and Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc. R. Soc. Lond. B*, **263**: 1003–1009.
- Gillespie, J.P., Kanost, M.R. and Trenczek, T. 1997. Biological mediators of insect immunity. *Annu. Rev. Entomol.*, **42**: 611–643.
- Grether, G.F., Hudon, J. and Millie, D.F. 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc. R. Soc. Lond. B*, **266**: 1317–1322.
- Johnsen, T.S. and Zuk, M. 1999. Parasites and tradeoffs in the immune response of female red jungle fowl. *Oikos*, **86**: 487–492.
- Kraaijeveld, A.R. and Godfray, H.C.J. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature*, **389**: 278–280.
- Kraaijeveld, A.R. and Godfray, H.C.J. 2001. Is there local adaptation in *Drosophila*–parasitoid interactions? *Evol. Ecol. Res.*, **3**: 107–116.
- Kurtz, J. and Sauer, K.P. 1999. The immunocompetence handicap hypothesis: testing the genetic predictions. *Proc. R. Soc. Lond. B*, **266**: 2515–2522.
- Kurtz, J., Wiesner, A., Götz, P. and Sauer, K.P. 2000. Gender differences and individual variation in the immune system of the scorpionfly *Panorpa vulgaris* (Insecta: Mecoptera). *Dev. Comp. Immunol.*, **24**: 1–12.
- Lively, C.M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. *Evolution*, **43**: 1663–1671.
- Lively, C.M. and Dybdahl, M.F. 2000. Parasite adaptation to locally common host genotypes. *Nature*, **405**: 679–681.
- Lively, C.M. and Jokela, J. 1996. Clinal variation for local adaptation in a host–parasite interaction. *Proc. R. Soc. Lond. B*, **263**: 891–897.
- Lochmiller, R.L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, **88**: 87–98.
- Møller, A.P. 1998. Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos*, **82**: 265–270.
- Møller, A.P. and Erritzoe, J. 1998. Host immune defence and migration in birds. *Evol. Ecol.*, **12**: 945–953.
- Møller, A.P. and Erritzoe, J. 2001. Dispersal, vaccination and regression of immune defence organs. *Ecol. Lett.*, **4**: 484–490.
- Møller, A.P., Christe, P., Erritzoe, J. and Møller, A.P. 1998. Condition, disease and immune defence. *Oikos*, **83**: 301–306.
- Morand, S., Manning, S.D. and Woolhouse, M.E.J. 1996. Parasite–host coevolution and geographic patterns of parasite infectivity and host susceptibility. *Proc. R. Soc. Lond. B*, **263**: 119–128.
- Moret, Y. and Schmid-Hempel, P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science*, **290**: 1166–1168.

- Norris, K. and Evans, M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav. Ecol.*, **11**: 19–26.
- Oppliger, A., Vernet, R. and Baez, M. 1999. Parasite local maladaptation in the Canarian lizard *Gallotia galloti* (Reptilia: Lacertidae) parasitized by haemogregarian blood parasite. *J. Evol. Biol.*, **12**: 951–955.
- Piersma, T. 1997. Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? *Oikos*, **80**: 623–631.
- Råberg, L., Nilsson, J.A., Ilmonen, P., Stjernman, M. and Hasselquist, D. 2000. The cost of an immune response: vaccination reduces parental effort. *Ecol. Lett.*, **3**: 382–386.
- Read, A.F. and Allen, J.E. 2000. Evolution and immunology – the economics of immunity. *Science*, **290**: 1104–1105.
- Reinhardt, K. 2001. Determinants of ejaculate size in a grasshopper (*Chorthippus parallelus*). *Behav. Ecol. Sociobiol.*, **50**: 503–510.
- Ryder, J.J. and Siva-Jothy, M.T. 2000. Male calling song provides a reliable signal of immune function in a cricket. *Proc. R. Soc. Lond. B*, **267**: 1171–1175.
- Ryder, J.J. and Siva-Jothy, M.T. 2001. Quantitative genetics of immune function and body size in the house cricket, *Acheta domesticus*. *J. Evol. Biol.*, **14**: 646–653.
- Sheldon, B.C. and Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.*, **11**: 317–321.
- Siva-Jothy, M.T. 2000. A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proc. R. Soc. Lond. B*, **267**: 2523–2527.
- Wade, M.J. 1990. Genotype–environment interaction for climate and competition in a natural population of flour beetles, *Tribolium castaneum*. *Evolution*, **44**: 2004–2011.

