10 Evolutionary Ecology of Insect Host–Parasite Interactions: an Ecological Immunology Perspective

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1. Introduction

Parasites and pathogens pose a ubiquitous threat to all organisms. However, until relatively recently, the impact of parasites on the ecology and evolution of their hosts had been largely ignored by biologists. Now, of course, parasites are recognized as an important selective force on their hosts and a key factor influencing their population dynamics (Hudson *et al.*, 2002; Grenfell and Dobson, 1995). The majority of studies examining the evolutionary ecology of host–parasite interactions have been conducted on vertebrate hosts, despite the fact that most animals are insects and other invertebrates. In recent years, though, this taxonomic bias has been challenged, as biologists have exploited the logistical advantages that insects and their parasites often provide.

The aim of this chapter is to review recent advances in our understanding of insect host–parasite interactions. The theoretical framework underpinning this work has largely come from the field of 'ecological immunology' (Sheldon and Verhulst, 1996; Rolff and Siva-Jothy, 2003; Norris and Evans, 2000; Schmid-Hempel, 2003), a new and growing field concerned with understanding the evolutionary ecology of parasite resistance mechanisms. Ecological immunology is about the ecological and evolutionary causes and consequences of variation in immunity, defined in its widest sense (see below), to include any mechanism that improves the capacity of an organism to resist a parasite or pathogen. Essentially, ecological immunology is examining the proximate and ultimate causes of variation in disease resistance, and it takes an evolutionary ecology approach. In the past, this field has been dominated by biologists working on vertebrate systems, particularly birds (Norris and Evans, 2000). However, there is a growing belief that because the insect immune system is relatively simple in comparison with that of vertebrates, in that it lacks a conventional acquired immune system, ecological immunology studies are likely to be most successful when applied to insects.

This chapter is divided into three main parts. In Section 2 we explore the diverse range of behavioural, physical and immunological defence mechanisms employed by insects to combat the threat that parasites pose. A fundamental assumption of life-history theory in general, and ecological immunology in particular, is that life-history and related traits (including immunity) are costly and result in physiological or genotypic trade-offs (Stearns, 1989, 1992; Roff, 2002). Therefore, in Section 3 we review the evidence for costs of resistance in insects. Finally, in Section 4, we examine three case studies from our own work in which the ecological immunology approach has been used to potentially gain new insights into the evolutionary ecology of insect host–parasite interactions.

2. A cascade of defence components

Over evolutionary time, organisms have evolved a suite of mechanisms that combine and interact to reduce the probability and impact of parasitic infections. Schmid-Hempel and Ebert (2003) refer to a 'cascade of defence components', to reflect the fact that the different resistance mechanisms act at different levels, in different sequences, and with different specificities. For example, there are behavioural mechanisms which generally reduce the probability of an animal encountering parasites in the first place; there are physical or physiological mechanisms which often reduce the probability that a given parasite will become established in its host upon contact; and there are immunological mechanisms which increase the probability that the parasite will be killed or its pathological effects will be reduced (Fig. 10.1). Thus, behavioural mechanisms are often the first line of defence against parasites and pathogens (but see below), whereas immunological mechanisms, *sensu stricto*, are usually the last.
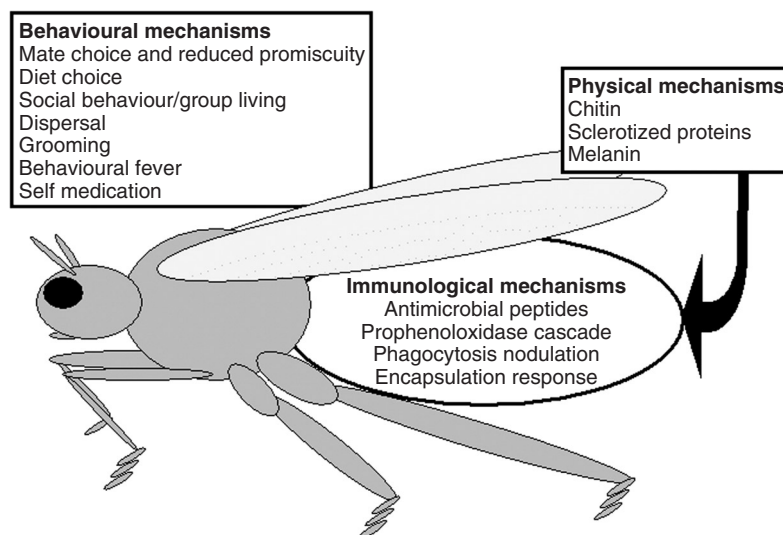

Behavioural mechanisms
Mate choice and reduced promiscuity
Diet choice
Social behaviour/group living
Dispersal
Grooming
Behavioural fever
Self medication

Physical mechanisms
Chitin
Sclerotized proteins
Melanin

Immunological mechanisms
Antimicrobial peptides
Prophenoloxidase cascade
Phagocytosis nodulation
Encapsulation response

Fig. 10.1. A cascade of defence components.

2.1 Behavioural mechanisms

There are a number of behavioural mechanisms that insects could employ to reduce their probability of becoming exposed to parasites and pathogens, and to ameliorate their effects should they become infected. These include: mate selection (to avoid parasites that are transmitted by potential sexual partners); selective diet choice (to avoid ingesting parasites); avoiding or dispersing from areas of high infection risk; social behaviours and group-living (to take advantage of 'dilution effects'); self-grooming and reciprocal allogrooming (e.g. to remove fungal spores or ectoparasites); behavioural fever and chills (i.e. altering body temperature to reduce parasite fitness and/or to increase immune function efficacy); and oral self-medication (i.e. ingesting nutrients that enhance physiological/immunological resistance mechanisms). Some of these behavioural mechanisms are reviewed here.

2.1.1 Mate choice and promiscuity

For parasites that are transmitted between sexual partners, there is an obvious benefit to being choosy or reducing levels of promiscuity. By avoiding mates with parasitic infections, an individual may reduce its probability of becoming infected. Whilst intuitively appealing, there is little evidence for mate choice in relation to infection status in insects. In a recent review of insect sexually transmitted diseases (STDs), Knell and Webberley (2004) reported finding only two examples where mate choice in relation to STD infection has been examined. Both studies involved beetles infected with mites: the leaf beetle, *Labidomera clivicollis*, infected with *Chrysomelobia labidomera* (Abbot and Dill, 2001), and the two-spotted ladybird, *Adalia bipunctata*, infected with the mite *Coccipolipus hippodamiae* (Webberley *et al*., 2002). In neither case was there any evidence that females discriminated between males with or without the sexually transmitted mite, suggesting that there was no mate choice in relation to the potential mate's infection status. Whilst at first this might seem puzzling, Knell (1999) has argued that avoidance of STDs is unlikely to be important in female choice, since there would be strong selection on STDs to become cryptic in such circumstances. Indeed, Graves and Duvall (1995) argued that STDs could select against mate choice in females because popular males might be more likely to infect them during mating (but see Boots and Knell, 2002; Kokko *et al*., 2002).

So, if selection favours STDs that avoid revealing themselves to potential new hosts, an alternative parasite-avoidance strategy might be simply to reduce the number of mates, so minimizing contact with potentially infective hosts (Hamilton, 1990; Sheldon, 1993; Loehle, 1995; Lockhart *et al*., 1996). Modelling studies suggest that STDs may select for reduced promiscuity, but if there is a fitness benefit to being promiscuous then this can result in a polymorphism, with some individuals being promiscuous and others being less so (Boots and Knell, 2002). At present, however, there is little evidence with which to test this prediction (Knell and Webberley, 2004).

Multiple mating might not only result in an increased risk of encountering an infected individual, but might also result in increased susceptibility to infection.

This is because a recent study on the mealworm beetle (*Tenebrio molitor*) has shown that, in both sexes, mating reduces the activity levels of phenoloxidase, a key enzyme in the insect immune system (Rolff and Siva-Jothy, 2002) (see below). This mating-induced downregulation of immunity lasts for at least 24 h and appears to be mediated by juvenile hormone. Thus, by mating frequently, individuals may be compromising their immune systems. Similar conclusions were reached in an earlier study by McKean and Nunney (2001) using the fruitfly *Drosophila melanogaster* (Fig. 10.2). They found that as the number of females housed with each male was increased (from 0 to 4), so there was a highly significant decrease in the male's ability to immunologically clear an experimental injection of *E. coli* bacteria. This reduced immunocompetence was not due to food shortage, since it was prevalent also when food was in excess. Nor was it simply due to crowding effects, since males housed with four males cleared the bacteria significantly faster than those housed with four females. Since both courtship behaviour and mating activity of males increase with the number of females available, it seems likely that one or both of these activities results in male immunosuppression. Thus, decreased immunocompetence may be a significant 'cost of reproduction' in insects.
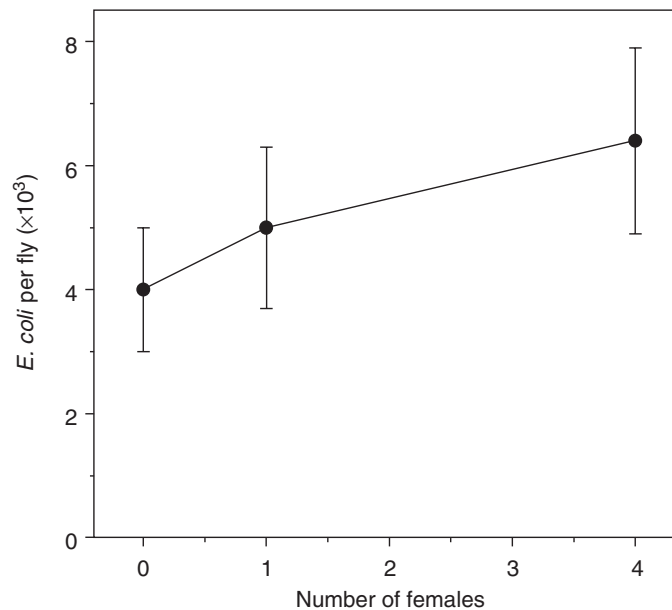

Fig. 10.2. Relationship between mating activity and immunity in the fruitfly, *Drosophila melanogaster.* The symbols (and bars) show the number of *E. coli* bacteria (±95% confidence interval) recovered from males kept in vials alone, or with either 1 or 4 virgin females that were replaced daily. As the number of females each male has access to increases, so his ability to clear an experimental infection of *E. coli* declines. Redrawn after McKean and Nunney (2001).

2.1.2 Parasite avoidance behaviours

It is not just sexually transmitted parasites that need to be avoided, others types of parasites do also. One strategy used by many vertebrates to reduce contact rates with directly transmitted macroparasites (e.g. nematodes) is to spatially segregate feeding and defecating areas (often by using specialized 'latrines'), thereby avoiding faeces contaminated with infective eggs or larvae (Hart, 1994; Hutchings *et al.*, 1998, 1999). Whilst we are not aware of any examples of such behaviour in insects, there are instances in which insects are known to avoid parasites directly (e.g. Evans, 1982; Hajek and St. Leger, 1994) or avoid dead conspecifics which might harbour such parasites (e.g. Kramm *et al.*, 1982).

Other avoidance patterns may be more subtle. For example, it appears that the foraging behaviour of the leaf-cutter ant, *Atta cephalotes*, is influenced by the presence of the diurnal parasitoid, *Neodohrniphora curvinervis* (Orr, 1992). Ants foraging above ground for leaves during the day are frequently parasitized by this day-flying phorid fly and so most ants forage at night instead. That this behavioural shift is a consequence of parasite pressure is indicated by the fact that when artificial lighting was supplied to allow phorids to hunt past dusk, ants foraged less than when light was provided but flies were removed (Orr, 1992). In this example, it appears that the ants were responding directly to the physical presence of the parasites. But in other instances, this may not be necessary to evoke avoidance behaviour. For example, in laboratory studies, it has been shown that gravid *Aedes aegypti* mosquitoes oviposit fewer eggs in water that contains conspecific larvae parasitized by the digenean parasite, *Plagiorchis elegans*, than in water that contains non-parasitized larvae (Lowenberger and Rau, 1994). More extraordinary though, they also dis-criminate against water that was previously home to parasitized larvae, even if this water was subsequently boiled, treated with antibiotics, or filter-sterilized. This suggests that *A. aegypti* females are deterred from ovipositing in response to chemical cues produced by parasitized larvae.

2.1.3 Self-grooming and allogrooming

In many social insects, self-grooming and allogrooming is extremely prevalent and may be an effective strategy for removing the spores of entomopathogenic fungi or the infective stages of entomopathogenic nematodes (Schmid-Hempel, 1998). In the dampwood termite, *Zootermopsis angusticollis*, allogrooming increases in frequency during and after exposure to the spores of the fungus *Metarhizium anisopliae* (Rosengaus *et al.*, 1998b). This appears to be effective in removing potentially infectious spores from the cuticle, so increasing termite survival. Thus, allogrooming plays a crucial role in the control of disease in termites, and Rosengaus and colleagues speculate that this advantage of group living may have been significant in the evolution of social behaviour in the Isoptera. Across ant species, the frequency of allogrooming tends to increase with colony size (Schmid-Hempel, 1998), whilst the frequency of self-grooming tends to do the opposite (Schmid-Hempel, 1990). In some ways, these results

appear counterintuitive. This is because, if parasites pressure is greater in larger colonies (as seems likely; Schmid-Hempel, 1998), then we might expect the rate of self-grooming to increase (to maximize hygienic behaviour) and the rate of allogrooming to decline (to minimize risk of exposure to pathogens). However, the relationship between group size and infection risk is not straight-forward and predictions regarding grooming behaviour are not easily made (e.g. Coté and Poulin, 1995; Wilson *et al*., 2003a; see below).

In vertebrates, it has been shown that grooming is a costly activity (Moore, 2002) and may be traded-off against vigilance behaviour (antelopes; Hart *et al*., 1992; Hart, 1992), resting (bats; Giorgi *et al*., 2001), thermoregulatory capacity (moose; Samuel, 1991), energy expenditure (bats; Giorgi *et al*., 2001) or even saliva production (rats; Ritter and Epstein, 1974). For example, in bats, the experimental inoculation of 20 or 40 ectoparasitic mites resulted in an increase in grooming activity and a concomitant decrease in resting activity, resulting in a drastic increase in overall metabolism (oxygen consumption) and weight loss (Giorgi *et al*., 2001). However, as far as we are aware, the costs of grooming have not yet been quantified in any insect species.

2.1.4 Feeding behaviour and self-medication

It is well now established that the impact of parasites on their hosts is often dependent on the quality and quantity of the host's diet (e.g. Duffey *et al*., 1995) and thus by choosing what (and how much) to eat, an animal is in a position to influence its exposure and susceptibility to parasitism. For example, the sus-ceptibility of gypsy moth (*Lymantria dispar*) larvae to its nucleopolyhedrovirus (GMNPV) varied significantly between four host-plant species, with the LD_{50} ranging from just 8000 viral occlusion bodies (OB) per larva when they were fed GMNPV on bigtooth aspen (*Populus grandidentata*) to more than 500,000 OB per larva when they were fed on black oak (*Quercus velutina*); this amounts to a 60-fold difference in susceptibility. In this particular example, decreased viral pathogenicity (increased larval survival) was correlated with increased acidity and hydrolysable tannin content of the leaves (Keating *et al*., 1988).

Differences in parasite virulence in relation to diet may be because the structural, physical or chemical properties of a particular food (e.g. host plant) alter parasite transmission; because the diet influences the host's parasite resistance mechanisms, and hence susceptibility to the parasite; or because of an interaction between these two factors. Determining the precise nature of the host–parasite–diet interaction is difficult, because these different chemical and physical components interact in ways that are often complex (Duffey and Stout, 1996). In the case of baculoviruses of insect herbivores like gypsy-moth larvae, it appears that leaf phenolics may present a chemical barrier to viral infection (e.g. Young *et al*., 1995). The precise mechanism by which this occurs remains to be established, but phenolic extracts from leaves that inhibit NPV activity cause the viral OBs to form large aggregations (though this does not appear to reduce their infectivity; Keating *et al*., 1990).

It is not just herbivorous insects that are affected by the properties of their diet. The malarial vector, *Anopheles stephensi*, requires both a recent blood

meal and a sugar source before it can successfully develop an effective melanization immune response (Koella and Sørensen, 2002). Thus, there is a significant interaction between dietary components. Similarly, a recent study of the Egyptian leafworm, *Spodoptera littoralis*, showed that resistance to NPV was critically dependent on the carbohydrate–protein balance of the diet, with insects that were fed on a high-protein, low-carbohydrate diet showing the highest survival following NPV challenge (Lee, 2002). Moreover, when insects were given *ad libitum* choice of two nutritionally complementary foods, those that survived virus challenge had selected a diet containing a higher protein content than those that had succumbed (or larvae that had not been challenged with NPV). It remains to be established, however, whether the larvae were engaging in nutritional 'self-medication' (i.e. actively seeking out a protein-rich diet in response to infection) or if there is a distribution of intake ratios within a population of caterpillars, and infection simply culled those larvae that had chosen a low-protein diet. Further studies are clearly required to distinguish between these possibilities.

2.1.5 Behavioural fever and chills

In mammals and other endotherms, parasitic infection is often associated with an endogenous fever response, in which the host's body temperature is increased above the norm by several degrees. It is usually argued that this is an adaptive response that helps the body to fight infection by stimulating natural defence mechanisms, though the issue remains controversial (Kluger *et al.*, 1998). Whilst it is true that under many circumstances the body's elevated temperature can help fight off an infection, this is not always the case, and excessive fever can cause problems such as dehydration. Demonstrating the adaptive value of fever in endotherms has proved problematic because reducing fever (e.g. by the administration of aspirin) usually has other major physiological effects such as pain reduction, reduced inflammation, and other responses that could help the animal to defend itself against parasites. Thus, isolating the effects of fever from these other responses is extremely difficult in endotherms.

Many ectotherms, including insects, also exhibit a fever response to infection. However, this response is behavioural rather than physiological, as found by Kluger *et al.* (1975), working on the desert iguana (*Dipsosaurus dorsalis*). Since then, similar experiments have been conducted on a range of insects and their parasites. For example, the North American grasshopper, *Melanoplus sanguinipes*, exhibits behavioural fever in response to infection by the lethal protozoan *Nosema acridophagus*. Grasshoppers kept at these fever temperatures survive longer and gain weight more rapidly than when kept at temperatures (6°C cooler) preferred by uninfected conspecifics (Boorstein and Ewald, 1987). However, fever is not shown in this same grasshopper species in response to infection by a closely related parasite, *Nosema locustae* (Hanley, 1989; cited in Ewald, 1994). Indeed, even when fever is elicited by a parasite, its effectiveness may depend critically on the susceptibility of the parasite to extreme temperatures. For example, when the clearwinged grasshopper, *Camnula pellucida*, is infected with the fungus *Entomopgaga grylli*, it generates

a fever in excess of 40°C. This fever response is capable of killing the US strain of the fungus, but not an Australian strain, which is resistant to high temperatures (Carruthers *et al*., 1992; Ewald, 1994).

Behavioural fever is a taxonomically widespread phenomenon and examples have been found in annelids, arthropods (including insects), fishes, amphibians and reptiles. Insect examples include: hissing Madagascar cockroaches (*Gromphadorhina portentosa*) infected with an *E. coli* suspension or an endotoxin (Bronstein and Conner, 1984); field crickets (*Gryllus bimaculatus*) infected with the lethal intracellular parasite, *Rickettsiella grylli* (Louis *et al*., 1986); the house cricket, *Acheta domesticus*, when infected with the same parasite, but not when infected with the bacterium *Serratia marascens* (Adamo, 1998); and the desert locust, *Schistocerca gregaria*, infected with the entomopathogenic fungus *Metarhizium anisopliae* (Blanford and Thomas, 1999; Wilson *et al*., 2002). However, behavioural fever is not a ubiquitous phenomenon (*e.g.* Ballabeni *et al*., 1995). In particular, there is little experimental evidence at present that macroparasites (nematodes, tapeworms, parasitoids, etc) induce behavioural fever in insect hosts (but see Karban, 1998).

Changes in thermal response in relation to parasitism are not restricted to behavioural fever, they may also be manifested in behavioural chills. For example, although foraging bumblebee workers (*Bombus terrestris*) normally return to the nest at night, those that have become parasitized by a conopid fly (Conopidae, Diptera) remain outside the nest at night, where the temperatures are cooler (Müller and Schmid-Hempel, 1993). Moreover, when given a choice of temperatures, parasitized worker bees spend more time in cold areas than do non-parasitized ones, and these colder temperatures have been shown to increase the survival rate of infected workers, and fewer parasitoids complete their development (Müller and Schmid-Hempel, 1993).

Of course, these changes in thermal behaviour in response to parasitism may not always be adaptive from the host's point of view. In some cases they may be selectively neutral (a simple by-product of the pathology of infection), whereas in others they may be adaptive manipulations of host behaviour by the parasites themselves (*e.g.* Maitland, 1994), although conclusively demonstrating parasite-manipulation of host behaviour is notoriously difficult (Moore and Gotelli, 1990; Poulin, 2000; Moore, 2002).

In the vast majority of instances in which changes in host thermal behaviour have been observed in parasitized hosts, it remains to be established to what extent the survival benefits of the behavioural change result from direct negative effects of temperature on the parasite (*e.g.* Starks *et al*., 2000); from indirect effects via changes in the host's immune response (*e.g.* Ouedraogo *et al*., 2003); or from interactions between these two effects. However, a recent study of the migratory locust, *Locusta migratoria*, suggests that fever temperatures may enhance haemocyte production and thus host immunity via phagocytosis (Ouedraogo *et al*., 2003). Moreover, an earlier study of a refractory strain of the malarial vector, *Anopheles gambiae*, showed that the ability of the mosquito to melanize a Sephadex bead (a response similar to that which results in the death of ingested *Plasmodium*) declined significantly as the environmental temperature increased from 24°C to 30°C (Suwanchaichinda

and Paskewitz, 1998). Thus, determining the relative importance of direct and indirect effects of temperature on the host–parasite interaction is a challenge for future studies of this phenomenon (Thomas and Blanford, 2003). Understanding the relationship between temperature and pathogen resistance is complicated still further by the fact that the response may also vary between host genotypes infected with the same pathogen. For example, Stacey *et al*. (2003) found that although two clones of the aphid, *Acyrthosiphon pisum*, showed changes in susceptibility to the fungal entomopathogen, *Erynia neoaphidis*, which mirrored changes in the pathogen's *in vitro* vegetative growth rate at different temperatures, two other clones exhibited responses that differed considerably from this expected response. Such interactions between genotype and temperature may have important implications for our understanding of disease dynamics in natural populations.

The extreme sensitivity of insects and their pathogens to temperature (and the insects' thermoregulatory response to infection) may have important implications for the use of entomopathogens in biocontrol (Ewald, 1994; Thomas and Blanford, 2003). Typically, biocontrol agents are chosen on the basis of their virulence to a chosen pest species, based on laboratory bioassays undertaken at one or a few constant temperatures. Yet, it is now known that for many of the fungi used in biocontrol (such as *Beauveria bassiana* and *Metarhizium anisopliae*, for example), the speed and magnitude of kill is critically dependent on the host's body temperature and that of its environment. As a consequence, a pathogen may cause rapid and extensive mortality to its host under some thermal conditions, but may be virtually benign under others (Thomas and Blanford, 2003). This is true not just for locusts and grasshoppers and their fungal pathogens, but also for a range of other host species (including flies, moths, leafhoppers and aphids) and many different types of parasite (including microsporidia, bacteria, viruses, rickettsia and nematodes). Thus, it is becoming increasingly apparent that future biocontrol studies will have to factor into their analyses the relative thermal sensitivity profiles (Thomas and Blanford, 2003) of both the insect they are trying to control and the potential biocontrol agents. It is only by understanding the potentially complex interactions between the host, its parasite and their shared environment that effective biocontrol strategies will be developed (Thomas and Blanford, 2003).

2.2 Physiological mechanisms

The first physical line of defence against most parasites and pathogens is the integument (the outer layer of the insect). It comprises the epidermis and the cuticle. The epidermis is one cell thick; the cuticle is a secretion of the epidermis and covers the whole of the outside of the body, as well as lining ectodermal invaginations, such as the trachea (Chapman, 1998). The inner region of the cuticle contains chitin, a polysaccharide. Hardening of the cuticle is primarily a consequence of cross-links between cuticular protein molecules so that they form a rigid matrix – this is a process known as tanning or sclerotization

(Chapman, 1998). The greater the proportion of proteins that become cross-linked, the greater the degree of sclerotization, and the more rigid the cuticle becomes. Hard, heavily sclerotized cuticles may be difficult for parasites to penetrate (Chapman, 1998). As the cuticle hardens, it also darkens. This darkening may be a consequence of quinone tanning, but it may also involve the polymerization of quinones to form melanin.

2.2.1 Melanism and parasite resistance

Melanin is a nitrogen-containing polymer. In the integument, it is either incorporated into granules or scattered throughout the cuticle (Hiruma and Riddiford, 1988). It has at least two properties that are likely to impact on potential pathogens. First, because it is a polymer, melanin is likely to strengthen cuticle and so improve its ability to act as a *physical barrier* to the penetration of parasites and pathogens that enter via the cuticle, such as fungi, bacteria and even parasitoids (St. Leger *et al*., 1988). Second, melanin is highly *toxic* to microorganisms and has potent antimicrobial activity (e.g. Montefiori and Zhou, 1991; Ourth and Renis, 1993; Sidibe *et al*., 1996; Ishikawa *et al*., 2000), probably because it binds to a range of proteins (e.g. Doering *et al*., 1999) and inhibits many of the lytic enzymes produced by microorganisms, including proteases and chitinases (Kuo and Alexander, 1967; Bull, 1970).

The antifungal properties of melanin are demonstrated nicely by two *in vitro* experiments conducted in the 1980s. Söderhäll and Ajaxon (1982) showed that when the crayfish-parasitic fungus *Aphanomyces astaci* was grown on agar, there was significant inhibition of fungal growth when the growth-medium contained melanin or any of its precursor quinones (e.g. 5,6-dihydroxyindole), but not L-dopa. Subsequently, St. Leger *et al*. (1988) showed that when larval cuticles of *Manduca sexta* were induced to melanize by suspending them overnight in L-dopa, they resisted fungal penetration nearly twice as long as non-melanized cuticles (dopa-melanized cuticles 72 h, non-melanized cuticles 40 h). Thus, melanin may enhance disease resistance in insects not only by improving the physical properties of the cuticle, but also by enhancing its chemical properties.

Recently, the association between cuticular melanization and resistance to parasites and pathogens that enter their hosts percutaneously has been examined more explicitly. Wilson *et al*. (2001) examined variation in resistance to an entomopathogenic fungus (*Beauveria bassiana*) and an ectoparasitic wasp (*Euplectrus laphygmae*) in two phase-polyphenic lepidopteran species (*Spodoptera exempta* and *S. littoralis*). In both of these species, the colour of the isolated larval cuticle varies from near-transparent to nearly black, due to the deposition of melanin granules in the cuticle (Fig. 10.3a–c). Wilson and colleagues found that, relative to non-melanic conspecifics, melanic *S. exempta* larvae melanized a greater proportion of the ectoparasitoid's eggs (Fig. 10.3d), and that melanic *S. littoralis* were more resistant to the entomopathogenic fungus (in *S. exempta*, the association between melanism and fungal resistance was non-significant, possibly because the fungal dose was too high; see Wilson *et al*., 2001, for details). Similarly, when larvae of the Oriental armyworm,

Mythimna separata, were percutaneously infected with the entomopathogenic fungus, *Nomuraea rileyi*, non-melanic larvae were substantially more susceptible than melanic ones (LC$_{50}$s: non-melanic = 4.9×10^7 OBs, melanic = 27.2 x 10^7 OBs; Mitsui and Kunimi, 1988).

In the mealworm beetle, *Tenebrio molitor*, cuticular colour varies from red-brown ('tan') to black, due to the combined effects of sclerotization and melanization of the cuticle (Thompson *et al*., 2002; and references therein). As with the lepidopteran examples above, mealworm beetles are more likely to develop darker cuticles under crowded conditions (see below), although the phenotypic response to population density is not as strong (Barnes and Siva-Jothy, 2000). Barnes and Siva-Jothy (2000) examined variation in susceptibility
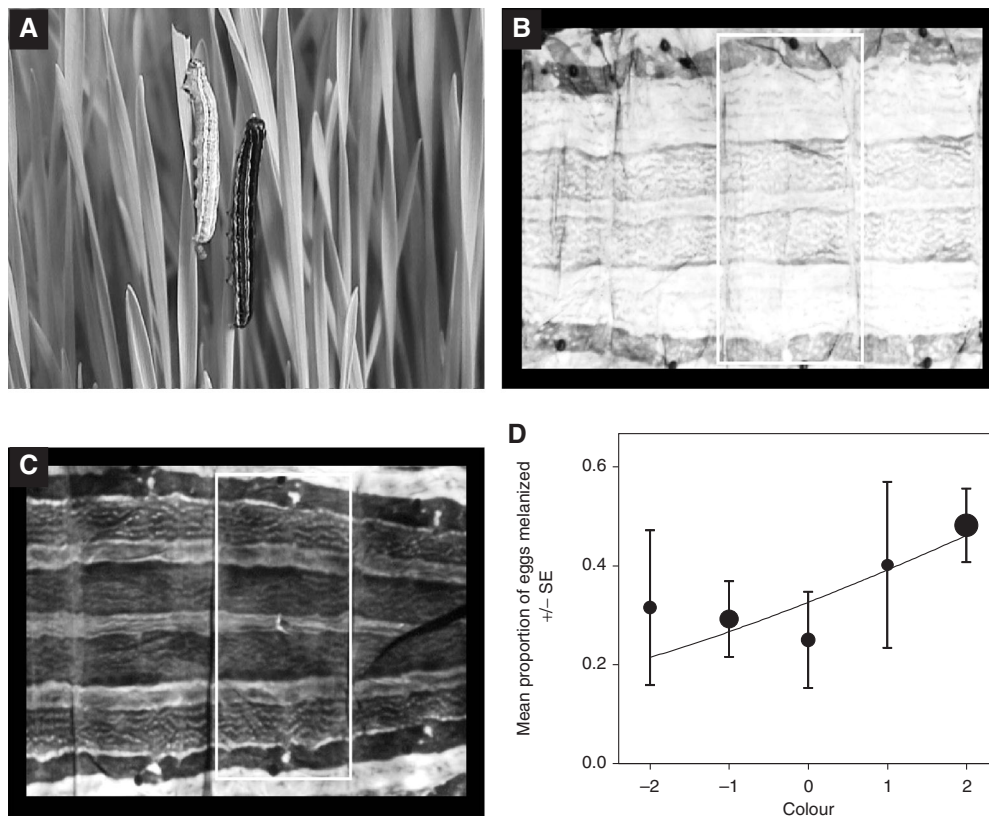

Fig. 10.3. Density-dependent cuticular melanization in *Spodoptera exempta*. **A** Live larvae, showing the pale, low-density phenotype on the left and the dark, high-density phenotype on the right; **B** the dorsal cuticle of the pale phenotype; **C** the dorsal cuticle of the dark phenotype; **D** relationship between cuticle colour and resistance to the ectoparasitoid, *Euplectrus laphygmae*. The vertical axis shows the proportion of melanized eggs (±SE) as a function of degree of cuticular melanization, scored on a scale from -2 (very pale) to $+2$ (very dark). Symbol size reflects sample size. The line is the fitted logistic regression to the raw data. Reprinted from Wilson *et al*. (2001).

to a generalist fungal pathogen (*Metarhizium anisopliae*) in relation to the cuticular colour of mealworm beetles and found that the highest mortality was observed in the palest (tan) beetles (91%) and the lowest in the darkest (black) beetles (29%).

Thus, the available evidence suggests that melanization and sclerotization of the cuticle is associated with greater resistance to parasites that enter their hosts via the cuticle. However, it is unclear, at present, how much of this effect is due to the physical and chemical properties of the cuticle and how much is due to correlated responses to aspects of the insect immune system (*see* below). Either way, the association between insect melanism and parasite resistance suggests that we may need to review our current understanding of the evolution of colour in insects. In fact, an association between melanism and disease resistance may not be restricted to insects (Owens and Wilson, 1999): recently, it has been suggested that similar correlations may also exist in vertebrate taxa including birds (Møller *et al.*, 1996; Gonzalez *et al.*, 1999; Evans *et al.*, 2000; Jawor and Breitwisch, 2003) and humans (Mackintosh, 2001).

2.3 Immunological mechanisms

One of the advantages of examining evolutionary questions about immunity using insects is that the insect immune system is simpler than its vertebrate equivalent, in that it lacks an adaptive or acquired immune system (in the conventional sense; but *see* below). There are many similarities between the vertebrate and invertebrate innate immune systems, and this makes insects potent models for understanding innate immunity (Vilmos and Kurucz, 1998). Even so, the insect innate immune system is still not fully understood and there remain gaps in our knowledge concerning the relative importance of its different components and how they interact. The immune defences of insects include constituitive and inducible defences, and both cellular and humoral components figure prominently. They include cell-mediated responses, such as nodulation, phagocytosis, and cellular encapsulation; the enzmes of the prophenoloxidase cascade; and inducible antimicrobial peptides (including lysozymes, attacins, cecropins and insect defensins). The description of the insect immune system given here draws heavily on that proposed by Gupta (2001a,b).

2.3.1 Cellular immune system

Insects produce several different types of haemocytes that can be distinguished to a large degree on morphological grounds, though these classifications have been the subject of intense debate. Gupta (2001a) identifies six cell types: granulocytes, plasmatocytes, spherulocytes, oenoctoids, adipohaemocytes and prohaemocytes (a stem cell which differentiates into the other morphotypes). Of these, only the granulocytes and plasmatocytes appear to participate in defence reactions, and are hence sometimes referred to as immunocytes (Gupta, 1991). These immunocytes are responsible for a number of cell-mediated defence

reactions, including phagocytosis, cellular encapsulation and nodulation, as well as the production and storage of the enzyme, prophenoloxidase (*see* below). In the Diptera, including *Drosophila melanogaster*, the lamellocytes take on these phagocytic and encapsulation roles, and prophenoloxidase is produced in the crystal cells.

Phagocytosis is a dynamic and energy-requiring defence reaction (Gupta, 2001a), in which bacteria and other small (<1 m diameter) particles are engulfed by the host haemocytes. This is followed by endocytosis and digestion of the foreign antigen in membrane-bound vesicles. Phagocytosis is usually associated with oxidative or respiratory burst and intracellular oxygen radical activity (Gupta, 2001a).

Encapsulation by immunocytes occurs when foreign abiotic (*e.g.* latex or Sephadex beads, nylon thread, etc) or biotic (*e.g.* bacteria, fungi, protozoa, nematodes or insect eggs and larvae) antigens are too large to be phagocytosed (Gupta, 2001a). Humoral (cell-free) encapsulation also occurs in some insects, especially in those insects that have a very low number of haemocytes (*e.g.* Diptera). Cellular encapsulation begins when, following random contact between immunocytes (granulocytes) and foreign antigens, the antigens are recognized by surface receptors on the immunocytes, which trigger exocytosis and the release of coagulation-, recognition-, growth- and opsonin-like factors, and/or phenoloxidase (*see* below). This is followed by the formation of 20–70 more layers of immunocytes (plasmatocytes) and the melanization of one or more layers of the cellular capsule (Gupta, 2001a). Melanization of the capsule appears to be the main mechanism for the killing or sequestering of most biotic and abiotic antigens via 'disinfectants' (Taylor, 1969). These probably include the highly toxic quinones produced during melanin production, but may also include other toxic molecules, such as nitric oxide and superoxide, released by cells localized in the innermost layers of the cellular capsule.

Although encapsulation is a highly effective defence mechanism against many parasites and pathogens, it is ineffective against some bacteria and parasitoids, which have evolved a range of strategies to inactivate or evade encapsulation. These strategies include: secretion of defensive membranes, exhibiting particular surface charge properties, molecular mimicry of host antigens, secretion or injection of anti-immune factors or particles, causing haemocytopaenia, and stimulating acquired immune tolerance in the host (*see* Gupta, 2001a, for details). For example, many hymenopteran endoparasitoids belonging to the Braconidae and Ichneumonidae inject polydnaviruses into their lepidopteran hosts during parasitization (*see* Beckage, 2003). These viruses are integrated into the genomic DNA of the wasp and undergo replication only in the female's ovary. When they are injected into the host during oviposition, they rapidly enter the host's haemocytes and the viral genes are expressed. The haemocytes then either alter their behaviour and fail to spread (so inhibiting the encapsulation response) or undergo fragmentation and programmed cell death (depending on the host and parasitoid species involved). Thus, it appears that the living parasitoid larvae either escape being detected as foreign by mechanisms that may involve host antigen mimicry or

masking, or by the presence of specific, and as yet unidentified, surface molecules that prevent their recognition as 'non-self' by immunocytes.

Nodule (or granuloma) formation involves both phagocytosis and encapsulation, and occurs in response to both inanimate and animate particulates (e.g. masses of bacteria) that are too numerous to be phagocytosed effectively. It is often difficult to distinguish between nodulation and encapsulation, as nodule formation also involves multicellular sheaths being formed by the immunocytes, but it appears that some of the encapsulating immunocytes detach from the nodule and enter the particulate mass (e.g. bacteria) to phagocyose them. Melanization of the nodule occurs in much the same way as in encapsulation. Nodulation can effect the production and release of antibacterial molecules from the fat body (the insect's functional analogue of the liver and the main source of circulating immune-related components; Christophides *et al.*, 2002).

Another important cellular process is coagulation and wound healing, which prevents any further loss of haemolymph following injury, and so maintains haemostasis. Wound healing is performed by granulocytes (sometimes referred to as coagulocytes). Recently it has been suggested that the frequency of wounding may be an important selective pressure influencing an organism's optimal investment in immune defences (Plaistow *et al.*, 2003). This is because using and maintaining an efficient immune system is costly (see below), and wound-healing and immunity share mechanisms and substrates in common. Thus, if the frequency of wounding is high, then many of the resources (haemocytes, phenoloxidase substrates, etc) that are utilized in healing the wound may not be available for immune function.

2.3.2 Humoral immune system

Whilst the immunocytes (granulocytes and plasmatocytes) provide the first line of defence against parasites and pathogens, via phagocytosis, encapsulation and nodule formation, during a prolonged microbial insult the cellular defences may become impaired due to immunocyte depletion. Under these circumstances, antimicrobial proteins secreted by the host's immunocytes and/or fat body provide a second line of defence (reviewed by Boman and Hultmark, 1987; Gupta, 1991, 2001b; Dunn, 1991; Hetru *et al.*, 1998). These include inducible antimicrobial peptides and polypeptides, agglutinins (lectins), cytokines ('factors'), neuropeptides and opioids, complement-like molecules, and the prophenoloxidase cascade (Gupta, 2001b).

Innate immunity in insects (as in mammals) is provided by various inducible antibacterial, antifungal and (presumably) antiviral proteins, which are produced in the haemocytes, fat body and epithelial tissues and are usually secreted into the plasma, where they act on the parasite either directly or via altering the behaviour of the immunocytes to enhance the immune response. Several hundred inducible antimicrobial peptides have now been described and these have been grouped into a number of types, including cecropins, attacins, insect defensins and lysozymes (for a review, see Faye and Hultmark, 1993). In the tobacco hornworm, *Manduca sexta*, injection of bacteria induced

more than 25 different proteins, including cecropins, attacins and lysozymes (Hurlbert *et al*., 1985).

The upregulation of antimicrobial peptide production takes time. For example, in the two giant silkworm moths, *Hyalophora cecropia* and *Antheraea pernyi*, the production of inducible antimicrobial peptides following bacterial challenge peaked at around 7–8 days post-infection, whereas in the smaller *Samia cynthia* it peaked in about half this time (Boman and Hultmark, 1987). After the peak, antimicrobial activity gradually declines, and disappears in about the same time as it took to reach the peak. In these particular examples, the antimicrobial peptides produced included attacins and cecropins.

Cecropins are a group of basic peptides (35–39 amino acid residues long) that affect the integrity of the cell membrane of most Gram-positive and especially Gram-negative bacteria, causing leakage of K^+ ions, interfering with ATP generation and ultimately causing cell lysis (Faye and Hultmark, 1993). Cecropins from species other than *Cecropia* have often been given new names, such as lepidopteran, bactericidin and sarcotoxin. However, given their obvious homology most are now referred to simply as cecropins. In each insect species, a family of cecropin forms is generally produced, and it is likely that each of these is adapted to the different needs of different compartments or developmental stages. For example, in *Drosophila*, cecropin A is mainly expressed in the fat body of larvae and adults, whereas the B and C forms appear to be preferentially expressed in the haemocytes of metamorphosing pupae (Samakovlis *et al*., 1990; Tryselius *et al*., 1992).

Insect defensins (such as sapecin) are cysteine-rich predominantly antibacterial peptides, named because of their apparent sequence similarity to the defensins produced from mammalian macrophages and neutrophils (Lehrer *et al*., 1991). In the meat fly, *Sarcophaga peregrina*, the defensins appear to be produced only by the haemocytes, whereas in the flesh fly, *Phormia terranovae*, they are synthesized also in the fat body (Faye and Hultmark, 1993). Like the cecropins and mammalian defensins, the insect defensins are believed to attack the bacterial cell membrane. Since insect defensins are most effective against Gram-positive bacteria, their antibacterial properties complement those of dipteran cecropins, which preferentially kill Gram-negative bacteria.

Recently, a number of peptides with defensin-like structures have been found to have potent antifungal properties, but no antibacterial activity. These include drosomycin (from *Drosophila melanogaster*; Fehlbaum *et al*., 1994; Michaut *et al*., 1996), heliomycin (from the tobacco budworm, *Heliothis virescens*; Lamberty *et al*., 1999), termicin (from the termite, *Pseudacanthotermes spiniger*; Da Silva *et al*., 2003), and gallerimycin (from the greater waxmoth, *Galleria mellonella*; Schuhmann *et al*., 2003). These antifungal peptides will provide the first line of defence against fungal pathogens that infect via the cuticle.

Attacins are a family of bactericidal proteins that affect only growing cells, by interfering with cell division. Their antibacterial spectra are rather narrow and only a few Gram-negative bacteria are killed with purified attacin (Hetru *et al*., 1998). The target of the attacins appears to be the biosynthesis of the outer membrane, which becomes permeabilized, and so may allow easier access for

lysozymes and cecropins. As with the cecropins, it appears that several different forms of these proteins may coexist within the same insect.

Lysozymes were the first antibacterial factors purified from insect haemolymph (Powning and Davidson, 1973). They are enzymes that degrade peptidoglycan in the bacterial cell wall. They are ubiquitous and are found not only in animals (including insects), but also plants, fungi and bacteriophages (see review by Jollès and Jollès, 1984). In insects, lysozymes are an important part of the immune defence. As with the other immune proteins, the main source of lysozyme in the haemolymph appears to be the fat body, although low levels are also found in the haemocytes. For example, in *Locusta*, lysozyme is stored in granulocytes and released during haemocyte coagulation. As well as the haemocoel, lysozyme is also found in the intestinal tract and, in *Manduca sexta*, it is specifically secreted there during metamorphosis (Russell and Dunn, 1991). In lepidopteran species (*Galleria*, *Bombyx*, *Spodoptera*, etc) lysozyme is induced in the haemolymph, along with other antibacterial factors, but it is also sometimes present at significant constituitive levels in non-induced insects. In *Drosophila*, and indeed most other non-lepidopteran insects, lysozyme does not seem to be induced at all by exposure to bacterial antigens (Faye and Hultmark, 1993). The lysozyme from *Cecropia* is bactericidal to only a few Gram-positive bacteria, and it has been suggested that its main function might not be to kill sensitive bacteria, but to clear up the debris left behind after the action of cecropins and attacins (Boman and Hultmark, 1987). In addition, it is likely that lysozyme works in synergy with cecropins and attacins (Engström *et al.*, 1985).

Agglutinins (lectins) are carbohydrate-binding proteins, produced in both the immunocytes and fat body, which agglutinate bacteria, protozoa and metazoan parasites, because of the presence of particular polysaccharides on their cell surface (Hetru *et al.*, 1998). Agglutinins have many functions: they act as surface receptors, opsonins, can cause the recruitment and proliferation of immunocytes during encapsulation, and are also involved in histogenesis and wound healing.

Specific antiviral (interferon-like) molecules have not yet been found in insects, despite antiviral resistance having been well-characterized in a number of field and laboratory populations (e.g. Boots and Begon, 1993; Abot *et al.*, 1995). It remains to be established whether specific antiviral molecules are waiting to be discovered.

2.3.3 Prophenoloxidase cascade

The enzymes of the prophenoloxidase cascade oxidize tyrosine and its derivatives to their corresponding quinones and their polymerization product, melanin (Mason, 1955; Hiruma and Riddiford, 1988; Nappi and Vass, 1993). These enzymes are involved not only in cuticular melanization (see above), but also in the various immune responses directed against parasites and pathogens, including cellular encapsulation, humoral (cell-free) encapsulation and nodule formation (Poinar, 1974; Gotz, 1986; Paskewitz *et al.*, 1988; Hung and Boucias, 1992; Beckage *et al.*, 1993; Washburn *et al.*, 1996, 2000).

The highly reactive enzyme phenoloxidase (PO) is synthesized and stored as an inactive zymogen called prophenoloxidase (proPO). Sequence data from a range of insect prophenoloxidase enzymes clearly indicate that insect proPO is homologous to arthropod haemocyanin, with the overall amino acid sequence homology being between 30% and 40% (Ashida and Brey, 1998). The exact localization of proPO and PO in insect haemolymph remains controversial. Leonard *et al*. (1985) argued that proPO is stored in granulocytes and released into the haemolymph by degranulation (exocytosis). However, Ashida and Yamazaki (1990) have argued that because the proPO genes lack a signal peptide signature, the proPOs are not released into the haemolymph by secretion but by rupture of haemocytes. Once in the haemolymph, the proPO is activated by a specific serine protease cascade (known as the prophenoloxidase cascade or the prophenoloxidase activating system). This cascade is triggered by minute amounts of microbial cell wall components, such as β-1,3 glucans (found in yeast and fungal membranes), and lipopolysaccharides and peptidoglycans (found in bacterial cell walls).

The insect cuticle also contains phenoloxidase enzymes (Lai-Fook, 1966; Hiruma and Riddiford, 1988), which have previously been referred to as wound phenoloxidase and granular phenoloxidase (Ashida and Yamazaki, 1990). It appears that wound (or cuticular) PO is synthesized in the haemolymph and transported to the cuticle, suggesting that the polypeptide backbone of the enzyme is likely to be identical to that of haemolymph PO (Ashida and Brey, 1995; Asano and Ashida, 2001). The relationship between haemolymph PO and granular PO is less clear, but Hiruma and Riddiford (1988) found that polyclonal antibody raised against granular PO did not cross-react with haemolymph proPO.

The midgut is an important site for resisting pathogens that enter the host orally, such as baculoviruses, protozoa and many bacteria, and phenoloxidase has often been implicated in midgut defence reactions. For example, the spread of baculovirus in non-permissive lepidopteran hosts appears to be blocked by aggregations of haemocytes that form melanotic capsules around infected cells in the midgut trachea (Washburn *et al*., 1996, 2000). In tsetse flies (*Glossina* spp.), there was a significant positive association between PO activity and refractoriness to the protozoan *Trypanosoma brucei rhodesiense*, both within species (male versus female *G. morsitans morsitans*) and among species (*G. m. morsitans* versus *G. palpalis palpalis*) (Nigam *et al*., 1997). And, in *Anopheles gambiae* mosquitoes selected for resistance to malaria, parasite ookinetes were melanized between the midgut epithelial cells and the basal laminae, completely blocking parasite transmission (Collins *et al*., 1986). These resistant insects also showed higher phenoloxidase activity in the midgut following exposure to the parasite, indicating that the melanotic encapsulation was under phenoloxidase control (Paskewitz *et al*., 1989). Recent studies on another *Anopheles* species (*A. stephensi*) indicate that the phenoloxidase isozyme isolated from the haemolymph differs from that extracted from the midgut (Sidjanski *et al*., 1997). However, as yet, the relative importance of the two isozymes in the melanization of malarial ookinetes remains to be established.

It is becoming increasingly apparent that most, if not all, insects possess not a single prophenoloxidase enzyme, but a number of functionally related

isoenzymes that may be activated in different tissues. For example, recent studies indicate that the genome of the mosquito *Anopheles gambiae* encodes for nine different proPO isoenzymes (Christophides *et al*., 2002), and both *Drosophila melanogaster* (De Gregorio *et al*., 2001) and the silkworm *Bombyx mori* (Yamamoto *et al*., 2003) produce three isoforms of proPO. However, regardless of how many different forms of the enzyme there are, all of the proPO enzymes are structurally similar, generate the same end-product (melanin), and are activated by similar combinations of substrates and triggers. It seems, therefore, that similar selection pressures are likely to prevail on all forms of the enzyme.

2.3.4 Molecular genetics of immune function

Recent advances in molecular genetics and the sequencing of the entire genomes of two important model insect species – *Drosophila melanogaster* (Adams *et al*., 2000) and *Anopheles gambiae* (Holt *et al*., 2002) – has allowed progress to be made in trying to understand the molecular genetic basis of immune function in insects (e.g. De Gregorio *et al*., 2001; Christophides *et al*., 2002).

Using high-density oligonucleotide microarrays encompassing almost the entire *D. melanogaster* genome, De Gregorio *et al*. (2001) examined the gene expression profile of adult flies in response to microbial infection, due to either septic injury with a mixture of Gram-negative (*Escherichia coli*) and Gram-positive (*Micrococcus luteus*) bacteria, or to a natural infection with an entomopathogenic fungus (*Beauvaria bassiana*). Out of 13,197 genes tested, they identified 230 genes that were induced following microbial infection and a further 170 that were repressed. Septic injury caused gene-expression changes in nearly all of the 400 *Drosophila* immune-related genes, whereas fungal infection regulated just 157, including one gene that responded only to *B. bassiana* infection, suggesting that it could be specific to this fungus (see below).

Much is now known about immune-related genes of *Drosophila*, and this can inform future studies of the molecular genetic basis of immunity in other insects of greater economic or health importance. Recently, Christophides *et al*. (2002) used this approach to conduct a comparative analysis of immune-related genes in *Anopheles* mosquitoes. They found that, relative to the genome as a whole, the immune systems of both species, and especially *Anopheles*, shows a deficit of well-conserved 1:1 orthologues and an overabundance of specific gene expansions with species-specific functions. In *Anopheles*, for instance, there are four genes that code for cecropins (effective against Gram-negative bacteria) and another four genes that encode insect defensins (most effective against Gram-positive bacteria). Thus, these important antimicrobial peptides are more numerous and more diverged than in *Drosophila* (Christophides *et al*., 2002). Similarly, as indicated above, there are nine genes coding for prophenoloxidase in *Anopheles*, but just three in *Drososphila*. Christophides and colleagues interpret these patterns as indicating that in many immune gene families, orthologues are under selection pressure to

diversify, or are lost, whereas certain immune genes reduplicate and then diversify. In other words, there is strong selection to adjust and expand the innate immune repertoire in response to new ecological and physiological conditions. In the case of *Anopheles*, these challenges include blood-borne infectious agents such as *Plasmodium*.

Of course, both *Drosophila* and *Anopheles* are dipterans and, as such, their immune systems differ in a number of important ways from many other insect groups (e.g. they lack an effective cellular encapsulation response, due to low haemocyte numbers). Therefore, one of the major challenges for future studies will be to determine the similarities and differences between the molecular genetics of immune function in dipterans and non-dipterans, especially lepidopterans, which include many of the most economically-important pest species. Another challenge will be to integrate whole-genome studies of insects with those of their parasites (e.g. *Autographa californica* and AcNPV) to gain new insights into insect host–parasite coevolution (Taylor *et al*., 2000).

2.3.5 Adaptive immunity and specific memory in innate immune responses

Acquired or adaptive immunity in vertebrates is characterized by immunological memory and specificity. Because invertebrates lack the T-cell receptors and immunoglobulins that mediate vertebrate adaptive immunity, it is generally assumed that their immune responses also lack specificity and memory (e.g. Lemaitre *et al*., 1997). With the advent of new molecular methods, however, these assumptions are being challenged. In the fruitfly, *D. melanogaster*, it has been shown that the genes encoding antibacterial and antifungal peptides are differentially expressed depending on whether the insect is challenged by Gram-negative bacteria, Gram-positive bacteria, or fungal pathogens (De Gregorio *et al*., 2001). Indeed, following natural infection by the entomopathogenic fungus, *Beauvaria bassiana*, only peptides with antifungal activity were expressed, indicating that the antimicrobial response shows some degree of specificity (Lemaitre *et al*., 1997; De Gregorio *et al*., 2001). However, the exact degree of specificity remains to be established. Certainly, there are a number of invertebrate studies suggesting that infection patterns in the wild are dependent on the match between host and parasite genotypes (e.g. Lively and Dybdahl, 2000; Carius *et al*., 2001), and there are a growing number of studies suggesting specific memory in invertebrate host–parasite interactions, arguing for specificity in host immune responses.

Kurtz and Franz (2003) examined the specificity of immunological memory in an invertebrate, the copepod *Macrocyclops albidus*, infected with one of its natural parasites, the tapeworm *Schistocephalus solidus*. Their experimental design involved exposing each copepod to three tapeworm larvae and then, 3 days later, exposing them to either three sibling parasites or to three unrelated parasites from a different sibling group. In both cases, the tapeworms used in the second challenge were fluorescently labelled so that they could be distinguished from the parasites used in the primary challenge. They found that when the copepods had prior exposure to related parasites, re-infection success was approximately 48%, whereas when they had previous experience of unrelated

parasites, the success rate was nearly 60%, indicating that this invertebrate is exhibiting specific immunological memory to its parasites.

Similar results were gained by Little *et al*. (2003), using two strains of the pathogenic bacterium, *Pasteuria ramosa*, infecting the water flea, *Daphnia magna*. They observed that there appears to be maternal transfer of strain-specific immunity in this crustacean following exposure to the bacterium. When *D. magna* females were exposed to one strain of the bacterium and their offspring were exposed to either the same strain (i.e. homologous challenge) or a different strain (i.e. heterologous challenge), the proportion of offspring that became infected was greater for the heterologous than the homologous challenge. This indicates that *D. magna* is exhibiting strain-specific immunity to this parasite. Little and colleagues also examined the overall fitness benefit of this maternal transfer of immunity, by determining the timing and magnitude of differences in the rate of offspring production. They found that when exposed to a homologous challenge, *D. magna* females started reproducing earlier and produced up to 21% more offspring than when they were exposed to a heterologous challenge. Moreover, these fecundity effects translate into a significant fitness benefit of maternally-transferred immunity, such that populations exhibiting this maternal effect would double in size, relative to populations lacking such an effect, in just 2–3 generations. The mechanisms underlying this phenomenon remain to be determined.

So far, there is evidence for transgenerational transfer of immunity in just one insect, the bumblebee, *Bombus terrestris*. Moret and Schmid-Hempel (2001) used a split-colony design in which most of the worker bees in the treated half of the colony were injected weekly with LPS in Ringer's solution to activate the immune system, and control workers in the other half of the colony were treated with Ringer's solution alone. As expected, they found that workers in the treated half of the colony showed higher antibacterial activity than those in the control half. However, their phenoloxidase activity levels were lower, suggesting a possible phenotypic trade-off between the two immune responses (see below). They also found that immune-challenged groups produced fewer queens and had lower reproductive output overall, indicating a possible cost of deploying the immune system (see below). Most significantly, perhaps, they found that male offspring from challenged groups showed higher phenoloxidase activity (and encapsulation response) than controls, although both haemocyte counts and antibacterial activity were comparable. Again, the mechanism by which this transgenerational transfer of immunity occurs remains to be determined.

All of these studies indicate that there may be greater levels of specificity and memory in the invertebrate immune response than had previously been assumed. The challenge for future studies is to determine the mechanisms by which these are achieved and their costs (Schmid-Hempel and Ebert, 2003).

3. Costs of resistance

An important assumption of the life-history theory approach to examining immune defence is that immunity is costly and is traded off against other fitness

traits (Sheldon and Verhulst, 1996; Owens and Wilson, 1999; Lochmiller and Deerenberg, 2000). This assumption is based on the idea that if there were no costs then there would be no penalty associated with maintaining or activating the immune system at its maximal level. The fact that we observe considerable genetic and phenotypic variation in immune function implies that there must be costs. There are three different kinds of costs that have been identified and are discussed below: deployment costs, maintenance costs and evolutionary costs (Table 10.1).

3.1 Deployment costs

The most obvious costs associated with parasite resistance are those that are incurred when the immune system (or other resistance mechanism, see above) is deployed in response to a parasitological challenge. In both vertebrates and invertebrates, there is now good evidence that using the immune system is costly (see review by Schmid-Hempel, 2003). For example, in *Drosophila melanogaster*, those flies that have successfully encapsulated an egg of the parasitoid *Asobara tabida*, have reduced fitness as adults (Fellowes *et al*., 1999c): capsule-bearing adults of both sexes are smaller than control flies, females produce significantly fewer eggs, and males allowed to copulate just once produce fewer offspring (though capsule-bearing males allowed repeated copulations with females do not show a reduction in fecundity). Thus, it appears that there is a trade-off between using resources in defence against parasitism and using those same resources in processes promoting fecundity and mating success. Similarly, when larvae of *Culex pipiens* mosquitoes were infected with the microsporidian *Vavraia culicis*, infected females pupated significantly earlier than uninfected females and tended to emerge as smaller adults, indicating a cost to their fecundity. However, the age and size at maturity of infected male mosquitoes was no different from uninfected males, indicating possible gender differences in the costs of resisting parasitic infection (Agnew *et al*., 1999; see below).

Whilst both of these studies suggest that immune defence against parasites is costly, it is unclear whether the observed costs are due to the host initiating an encapsulation response; to the pathological damage caused by the parasite; or due to a combination of the two. Thus, the only way to reliably determine the magnitude of the costs associated with deploying the immune system is to measure the costs in the absence of the parasite. For example, in insect studies this has often involved triggering an immune response with Sephadex beads, a nylon implant, or a microbial cell wall component (e.g. lipopolysaccharide; LPS), and comparing the subsequent fitness of these individuals with control insects whose immune systems have not been activated. Because the immune elicitors are non-pathogenic and non-living, yet trigger the invertebrate immune system, the costs of deploying the immune system are isolated from the pathological costs associated with the parasites themselves.

One of the best examples of this approach is a recent study by Moret and Schmid-Hempel (2000) working with the bumblebee, *Bombus terrestris*. They

Table 10.1. Costs of resistance in insects. Adapted after Schmid-Hempel (2003).

Type of cost	Species	Protocol	Effect	Reference
Deployment costs (effects of immune challenge on fitness traits)	Captive bumble bee (*Bombus terrestris*)	Antigenic challenge by injection of LPS and Sephadex beads	Reduced survival only when starved	Moret and Schmid-Hempel, 2000
	Cabbage white butterfly (*Pieris brassicae*)	Pupae challenged with nylon implant	Standard metabolic rate of challenged pupae raised by nearly 8%	Freitak *et al.*, 2003
	Mosquito (*Armigeres subalbatus*)	Experimental infection with microfilariae taken from mammalian host	Infection reduces egg development	Ferdig *et al.*, 1993
	Mosquito (*Culex pipiens*)	Experimental infection with a microsporidian (*Vavraia culicis*)	Infected females pupate earlier and emerge as smaller adults, but no effect on males	Agnew *et al.*, 1999
	Fruitfly (*Drosophila melanogaster*)	Experimental infection by larval parasitoid (*Asobara tabida*)	Infected females smaller as adults and less fecund; adult males smaller and less fecund if allowed to mate just once. Puparial walls thinner	Fellowes *et al.*, 1998b, 1999c
Deployment costs (effects of work, mating or resource limitation on immune function)	Captive bumble bee (*Bombus terrestris*)	Restricted access to food	Reduces reproductive success but has no effect on encapsulation response	Schmid-Hempel and Schmid-Hempel, 1998
	Free-flying bumble bee (*Bombus terrestris*)	Clipping wings to prevent foraging and flying	Foraging bees show reduced encapsulation response	König and Schmid-Hempel, 1995 Doums and Schmid-Hempel, 2000
	Wild damselfly (*Matrona basilaris*)	Observation of activity	After copulation or oviposition, encapsulation response decreased	Siva-Jothy *et al.*, 1998
	Mosquito (*Anopheles gambiae*)	Restricted larval access to food	Encapsulation response weaker in adults that had been nutritionally deprived	Suwanchaichinda and Paskewitz, 1998

	Species	Method	Result	Reference
	Fruitfly (*Drosophila melanogaster*)	Variation in access to receptive females	Ability to clear experimental injection of *E. coli* negatively related to mating activity	McKean and Nunney, 2001
	Mealworm beetle (*Tenebrio molitor*)	Short-term starvation	Phenoloxidase activity reduced (but not cellular encapsulation response)	Siva-Jothy and Thompson, 2002
	Mealworm beetle (*Tenebrio molitor*)	Comparison of experimentally mated and non-mated beetles	Mating reduces PO activity through juvenile hormone	Rolff and Siva-Jothy, 2002
Maintenance costs	Leaf-cutting ant (*Acromyrmex octospinosus*)	Experimental closure of paired exocrine metapleural glands	Reduced production of antimicrobial peptides leads to reduced energy expenditure	Poulsen *et al.*, 2002
	Mealworm beetle (*Tenebrio molitor*)	Observation of relationship between cuticular melanism and patterns of sperm precedence	Melanic males showed reduced sperm precedence but probably with low fitness consequences	Drnevich *et al.*, 2002
	Egyptian leafworm (*Spodoptera exempta*)	Observation of relationship between cuticular melanism and larval fitness	Melanic larvae smaller, have lower haemolymph protein levels and reduced lifespan	Cotter *et al.*, 2004a
	African armyworm (*Spodoptera exempta*)	Observation of relationship between cuticular melanism and larval and adult fitness	Melanic moths smaller, but produce more eggs when fed on sucrose solution	Mensah and Gatehouse, 1998
Evolutionary costs	Mosquito (*Aedes aegypti*)	Selection on age at pupation (and hence reproduction)	Earlier reproduction correlated with lower encapsulation response	Koella and Boëte, 2002
	Mosquito (*Aedes aegypti*)	Selection for increased resistance to nematode infection	Increased resistance correlated with reduced reproductive success	Ferdig *et al.*, 1993
	Fruitfly (*Drosophila melanogaster*)	Selection for increased encapsulation response to common larval parasitoid (*Asobara tabida*)	Increased resistance correlated with reduced larval competitive ability	Kraaijeveld and Godfray, 1997

Continued

Table 10.1. — *Continued.*

Type of cost	Species	Protocol	Effect	Reference
Evolutionary cost *Continued*	Fruitfly (*Drosophila melanogaster*)	Selection for increased encapsulation response to virulent larval parasitoid (*Leptopilina boulardi*)	Increased resistance correlated with reduced larval competitive ability	Fellowes *et al.*, 1998a
	Honeybee (*Apis mellifera*)	Selection for increased resistance to bacterial disease	Increased resistance correlated with higher larval mortality	Sutter *et al.*, 1968
	Honeybee (*Apis mellifera*)	Selection for increased resistance to bacterial disease	Increased resistance correlated with higher larval mortality	Rothenbuhler and Thompson, 1956
	Indian meal moth (*Plodia interpunctella*)	Selection for increased resistance to granulosis virus	Increased resistance correlated with slower development, lower egg viability, but increased pupal mass	Boots and Begon, 1993
	House cricket (*Acheta domesticus*)	Quantitative genetics of immunity and body size	Positive genetic correlations between haemocyte load, encapsulation response and body size in males; no evidence for costs	Ryder and Siva-Jothy, 2001
	Egyptian leafworm (*Spodoptera littoralis*)	Quantitative genetics of immune function and life history traits	Negative genetic correlations between antibacterial (lysozyme-like) activity and haemocyte density, haemolymph phenoloxidase activity and cuticular melanization	Cotter *et al.*, 2004b
	Velvetbean caterpillar (*Anticarsia gemmatalis*)	Selection for increased resistance to nucleopolyhedrovirus	Increased resistance correlated with lower larval survival rates, lower pupal weights, longer life spans, and reduced fertility	Fuxa and Richter, 1998

found that when they activated the bumblebee immune system by injecting individuals with LPS and/or small, sterile micro-latex beads (4.5 ˇmm diameter), the immune system was activated (as indicated by an increase in antibacterial activity, measured by a zone-inhibition assay). Moreover, the survival time of worker bees that had been injected with LPS and/or beads was reduced by factor of 1.5–1.7 (odds ratios) relative to control bees that were injected with Ringer solution alone (Fig. 10.4). Importantly, however, this cost of deployment was revealed only in worker bees that were starved post-challenge; activation of the immune system had no measurable cost to worker bees that were allowed to feed on sugar-water *ad libitum*. This result demonstrates that deploying immune defence mechanisms is costly in this species, but that these costs may be ameliorated by compensatory feeding to replenish the limiting resources lost to the immune system. An important implication of this observation is that, provided sufficient resources are available in the wild, the costs of immune activation may be insignificant under field conditions. Alternatively, given that it is probable that resources are more constrained under field conditions than in the laboratory, it suggests that the fitness costs of deploying the immune system may be extremely important in wild insect populations. It is therefore extremely important to determine the magnitude of nutrient constraints in the wild.
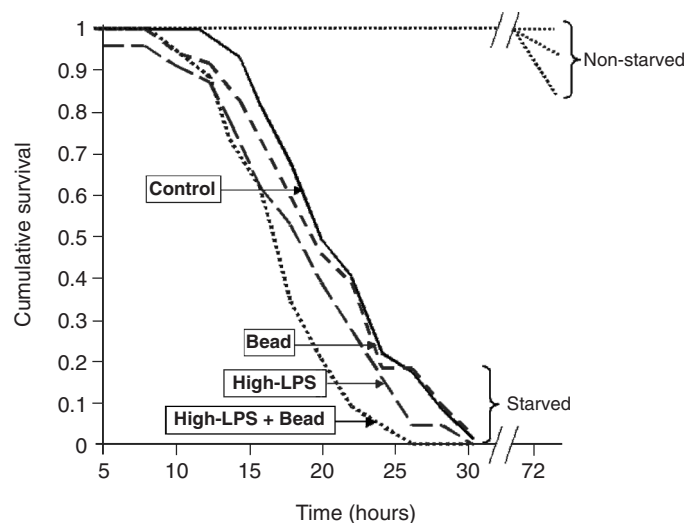

Fig. 10.4. Effect of diet and immune challenge on survival of the bumblebee, *Bombus terrestris.* Worker bees were either starved or fed sugar-water *ad libitum*, and were challenged with lipopolysaccharides (LPS), Sephadex beads, both, or neither. The top, nearly horizontal, lines refer to non-starved animals (no difference was found among treatments). High-LPS + beads refer to the combined injection of a high dose of lipopolysaccharides and beads. Cox regression analysis for the starved animals (sloping lines) showed that worker survival rate was reduced by the injection of Sephadex beads (odds ratio, OR = 1.56) or LPS (high dose, OR = 1.75). The addition of beads to LPS had an additive survival cost compared with LPS and beads alone. Redrawn after Moret and Schmid-Hempel (2000).

Another take-home message from this study is that resistance to parasites is likely to be condition-dependent. In other words, hosts with higher levels of the key limiting resources should be better able to fight off parasitic infections. In fact, there is good evidence for this prediction from another study of *B. terrestris* (Brown *et al.*, 2000). This showed that when worker bees had *ad libitum* access to pollen and sugar-water and were infected with an intestinal trypanosome (*Crithidia bombi*) the bees suffered negligible mortality rates (<6%), which did not differ from those of conspecifics in a control group that were not infected with the parasite (<5%). However, when workers were starved, the mortality rate of bees in the infected treatment group was approximately 1.5 times higher than that of bees in the control group. Thus, the virulence of the parasite was enhanced under conditions of host food stress.

Whilst these results suggest that parasite resistance is condition-dependent, the experimental protocol employed in both of these experiments is rather extreme (though they may mimic the situation faced by many insects when foraging is prevented for long periods by rain and/or cold weather; Brown *et al.*, 2000). It remains to be seen whether such effects are also observed under more realistic conditions, in which resources are constrained but not withdrawn completely. However, two studies indicate that such effects will prevail: Suwanchaichinda and Paskewitz (1998) found that when a *Plasmodium*-refractory strain of the mosquito, *Anopheles gambiae*, was well-nourished during the larval stages, they produced stronger encapsulation responses as adults than those that were nutritionally deprived. Similarly, Siva-Jothy and Thompson (2002) observed that in the mealworm beetle, *Tenebrio molitor*, phenoloxidase activity (but not cellular encapsulation) was downregulated in individuals that had experienced short-term starvation relative to conspecifics that were given *ad libitum* access to food.

All of the studies outlined above measured the costs of immune deployment by activating the immune system and measuring the fitness costs. Another way of demonstrating that immunity is costly is to increase the demands of other fitness components (e.g. by increasing workload) and to measure the impact on immune function. For example, when worker bumblebees (*Bombus terrestris*) were prevented from engaging in energetically demanding foraging activity by cutting their wings, their encapsulation response against a novel antigen (a nylon implant) was significantly stronger than that of conspecifics that were allowed to forage (König and Schmid-Hempel, 1995; Doums and Schmid-Hempel, 2000). Reproductive activity may also be costly. For example, the encapsulation response of Japanese calopterygid damselflies (*Matrona basilaris japonica*) was significantly reduced following copulation or oviposition (Siva-Jothy *et al.*, 1998); the ability of male fruitflies (*Drosophila melanogaster*) to clear an experimental injection of *E. coli* was significantly negatively related to the number of receptive females they had access to (McKean and Nunney, 2001; see above); and phenoloxidase activity (but not haemocyte load) was significantly reduced in mealworm beetles (*Tenebrio molitor*) following mating (Rolff and Siva-Jothy, 2002).

A key question that needs to be addressed in future studies is what are the key resources limiting immune expression? Although it is often assumed that immunity is *energetically* costly, only a few studies on vertebrates have detected

a change in energy budget as a consequence of immune stimulation (Lochmiller and Deerenberg, 2000). These suggest that when the vertebrate host mounts an immune response following vaccination or sepsis, the metabolic rate typically increases by around 15–30% (relative to resting metabolic rate). In the leaf-cutting ant, *Acromyrmex octospinosus*, experimental closure of paired exocrine metapleural glands prevented the production of a highly effective cocktail of antibacterial and antifungal peptides (Poulsen *et al*., 2002). Consequently, there was a significant reduction in the respiration rate of treated ants, suggesting that metapleural gland secretion incurs a substantial cost, though these costs may best be referred to as maintenance rather than deployment costs, because they are presumably paid even in the absence of the pathogen (see below).

As far as we are aware, only one study has examined the metabolic costs of mounting an immune response in an insect. Freitak *et al*. (2003) showed that when diapausing cabbage white butterfly (*Pieris brassicae*) pupae were challenged with a nylon implant, they raised their standard metabolic rate by nearly 8% relative to controls. In contrast, Alleyne *et al*. (1997) found that when tobacco hornworm (*Manduca sexta*) larvae were infected by the braconid wasp, *Cotesia congregate*, their metabolic rates decreased 1 day following parasitization. However, as Freitak and colleagues point out, the polydnaviruses (or venom) that accompany wasp eggs are known to temporarily suppress the host's cellular immune response (Strand and Pech, 1995; Shelby and Webb, 1999; Shelby *et al*., 2000), and so the lower metabolic rate of parasitized larvae may be a result of their reduced investment in immune defence. These two experiments demonstrate the importance of distinguishing between the costs of deploying the immune system from the costs associated with parasitism *per se*. Clearly, more studies are required before we can establish whether the metabolic cost of immune activation demonstrated in cabbage white butterfly pupae is typical of insects as a whole.

In insects, available *nitrogen* is often considered to be the major limiting resource (McNeill and Southwood, 1978; Crawley, 1983), and certain key *nutrients*, such as salt, may be essential for normal growth and survival of some species (Trumper and Simpson, 1993). However, the nutritional requirements for growth, reproduction and survival may be very different from those for an effective immune response. There is therefore a pressing need to identify the key macro- and micronutrients constraining insect immune function (but see Lee, 2002).

Given that the dietary precursors of melanin are phenylalanine (an essential amino acid) and tyrosine (a non-essential amino acid), it seems possible that these may constrain aspects of insect immune function (Johnson *et al*., 2003). Experimental evidence in support of this idea comes from a study of *Armigeres subalbatus*, the vector for the filarial worm, *Brugia malayi*, which is responsible for elephantiasis (Ferdig *et al*., 1993). This mosquito has a very effective encapsulation response, which can kill more than 80% of the microfilarial infective stages of its parasite within 36 h of ingestion. Tyrosine is required both for melanotic encapsulation and for egg-chorion tanning (amongst other things). Thus, because the blood meal both initiates egg development and is the source of the parasite, Ferdig and colleagues examined the possibility that the process

of encapsulating microfilaria in an infected blood meal would impose a reproductive cost on the host. As predicted, relative to control females, insects that had fed on an infected bloodmeal took significantly longer to develop their ovaries and to start oviposition. Moreover, normal processes of egg development, including vitelline accumulation, were drastically altered, and the tyrosine and total protein levels in their ovaries were less than half those of the controls. Another possible example comes from the *D. melanogaster–A. tabida* system, where the pupae of larvae that have successfully encapsulated a parasitoid have relatively thinner puparial walls (Fellowes *et al.*, 1998b). Since both encapsulation and puparium formation utilize the substrates and enzymes of the prophenoloxidase cascade, this is a likely mechanism linking these two phenomena. Regardless of the precise mechanism, the thinner walls of the puparium appear to impose a significant fitness cost, since the pupal parasitoid, *Pachycrepoideus vindemiae* preferentially attacks *Drosophila* pupae that have previously survived attack by the larval parasitoid, presumably because the thinner puparium reduces the handling time required for successful attack (Fellowes *et al.*, 1998b). Clearly, further studies are required in which important nutritional components are manipulated directly and their impacts on immune function in insects examined.

Another possibility is that the costs of resistance are mediated not by limiting resources but via physiological constraints. In the case of the downregulation of phenoloxidase activity exhibited by mealworm beetles (*T. molitor*) following mating, it appears that the costs of mating are mediated by juvenile hormone (JH), secreted from the *corpora allata* (Rolff and Siva-Jothy, 2002). Mating-induced JH secretion functions to switch on physiological processes associated with gametogenesis and spermatophore production (Wigglesworth, 1965), processes vital to both female and male fitness. It appears that a side-effect of this is that phenoloxidase activity is reduced, though the mechanism underlying this physiological trade-off remains to be elucidated. The immunological costs of mating detected in earlier studies on damselflies (Siva-Jothy *et al.*, 1998) and fruitflies (McKean and Nunney, 2001) may also be a consequence of similar physiological antagonisms.

A hitherto underrated potential cost of activating an immune response is the risk of self-reactivity or auto-immunity. This seems particularly likely in invertebrates, with their open circulatory system which exposes all of the host's vital organs to the immune effector systems it switches on in response to the recognition of non-self. When the prophenoloxidase cascade is activated, during melanotic and cellular encapsulation, a number of cytotoxic by-products are produced, including quinones and reactive oxygen species (see above). Whilst these may be extremely effective in combating the parasite, because of the open haemocoel, they may also cause significant damage to the host tissues if not rapidly detoxified (Nappi and Vass, 1993; Nappi *et al.*, 1995; Sugumaran *et al.*, 2000). The magnitude and frequency of this autoreactive threat remains to be determined. However, it has been argued that immune responses may be actively suppressed by animals under conditions when their risk of self-reactivity is high (Råberg *et al.*, 1998; Westneat and Birkhead, 1998).

3.2 Maintenance costs

Even when the immune system is not being used to combat a parasite or pathogen, insects that maintain high levels of immunological 'readiness' are still likely to pay significant costs. The distinction between deployment and maintenance costs has been likened to the respective penalties of fighting a war (deployment costs) and maintaining a standing army (maintenance costs) (Fellowes and Godfray, 2000; Wilson, 2001). Some of the costs associated with maintaining an effective immune system will be evolutionary costs (see below), with different genotypes expressing different constituitive levels of immune investment. However, within the constraints imposed by their genetic make-up, individuals will also exhibit phenotypic plasticity with respect to levels of prophylactic immune investment (Wilson and Reeson, 1998), and so are likely to incur phenotypic costs associated with physiological trade-offs.

In a number of recent studies of insects, it has been shown that immune function is upregulated in response to cues associated with high population density (e.g. Reeson *et al.*, 1998, 2000; Barnes and Siva-Jothy, 2000; see below for details). Similarly, in rodents, it has been shown that immune function is seasonally upregulated at the start of winter (Nelson and Demas, 1996; Nelson *et al.*, 1998) and, in humans, it appears that immune function is downregulated in high-performance athletes relative to non-athletes (Kumae *et al.*, 1994). Measuring the costs of immune maintenance in these and similar studies is inherently difficult because of the way in which the immune system is integrated with other physiological systems (Lochmiller and Deerenberg, 2000; Schmid-Hempel, 2003).

The costs associated with investment in prophylactic resistance have been measured in several insect species. In adult mealworm (*T. molitor*) beetles, individuals with cuticles that are dark and melanized are better able to survive exposure to fungal pathogens relative to individuals with pale, less melanized cuticles (Barnes and Siva-Jothy, 2000; see above). Thus, it is assumed that melanism incurs a marginal cost in the absence of the pathogen (otherwise selection would favour individuals always possessing a fungus-resistant, melanized cuticle). Drnevich *et al.* (2002) examined the relationship between male colour and sperm competitive ability and found that although dark males did lose sperm precedence over time relative to light males, this was unlikely to result in lower fitness for darker males under normal female remating frequencies. It remains to be seen whether there are other costs associated with being melanic in this species.

In the Egyptian leafworm, *Spodoptera littoralis*, cuticular melanism is also associated with resistance to an entomogenous fungus (Wilson *et al.*, 2001; see above). In this species, the melanic phenotype is smaller, has lower haemolymph protein levels and dies at a significantly earlier age (Cotter *et al.*, 2004a). Thus, there do appear to be small, but detectable, costs of maintaining high levels of investment in immune function. In contrast, in a related species, the African armyworm, *Spodoptera exempta*, the costs are less obvious. In this species, resistance to a range of entomopathogens (including nucleopoly-hedrovirus and an ectoparasitoid) is positively associated with larval density

and melanism, and the melanic, high-density phenotype has significantly higher levels of phenoloxidase activity than the non-melanic, low-density phenotype (Reeson *et al*., 1998, 2000; Wilson and Reeson, 1998; see below). Contrary to expectation, females raised under high-density conditions laid approximately 26% more eggs than those reared at low density when they were fed only water as adults (there was no phase difference in fecundity when the adult moths were fed on sucrose solution) (Mensah and Gatehouse, 1998). This is despite the fact that low-density females were significantly smaller, and fecundity tends to increase with body weight in this species (B.A. Mensah, unpublished). Thus, under laboratory conditions at least, high-density, melanic females do not appear to incur a fecundity cost to investing in immune function. However, in the wild, their lower body mass may impose a survival cost that is not evident in the laboratory. Moreover, the costs of melanism may not be physiological, but ecological, since melanic larvae may be more conspicuous and so suffer greater predation rates.

Thus, it appears that the phenotypic costs of maintaining an efficient immune system are sometimes evident, but they may not be large. In their review of the costs of immunity in mammals, Lochmiller and Deerenberg (2000) argued that the immune system may be analogous to the female reproductive system, where the costs of maintenance are minor in comparison with the costs of actually using it. Further studies on insects will be required before we are in a position to be able to say whether the same applies to insects.

3.3 Evolutionary costs

Evolutionary costs of resistance arise when the alleles associated with enhanced resistance have negative pleiotropic effects on other important fitness traits, such as growth or reproduction (Stearns, 1989, 1992; Roff, 2002), such that high levels of immunity can be expressed only at the expense of other life-history traits. As a result of these negative genetic covariances, individuals are constrained in the decisions they can make.

A number of methods have been used to characterize possible evolutionary costs of immunity in insects. These include: micro-evolutionary experiments (e.g. Boots and Begon, 1993), artificial selection experiments (e.g. Kraaijeveld and Godfray, 1997; Fellowes *et al*., 1998a), and breeding studies (e.g. Ryder and Siva-Jothy, 2001; Cotter *et al*., 2004b).

Boots and Begon (1993) took a novel approach to examine the genotypic costs of resistance in the Indian meal moth (*Plodia interpunctella*). They set up six replicate populations of the moth in small boxes. In three of these, they added a granulosis virus (GV) infection and, after 2 years, compared these populations against the three virus-free control populations. Based on LD_{50} bioassays with the GV, they found that moths from the virus-selected populations were nearly twice as resistant to infection as moths derived from the virus-free control populations, indicating that selection for increased viral resistance had indeed occurred. More significantly, they found that this increase

in resistance was correlated, at the population level, with a lengthening of the larval development time, a reduction in egg viability, and an increase in pupal weight. These changes in life-history traits resulted in a fitness cost of resistance of around 15%. Subsequently, it was argued that the reduced fitness of moths from the selected populations may have been due to fitness costs associated with maintaining a covert infection of the virus, rather than the costs of evolving virus resistance (Goulson and Hauxwell, 1995). Although it is now recognized that covert baculovirus infections may be highly prevalent in field and laboratory populations of Lepidoptera (e.g. Burden *et al.*, 2003), it seems unlikely that the magnitude of their effects on fitness are large enough to account for the results from this micro-evolutionary experiment (Begon and Boots, 1995).

Conventional artificial selection experiments using insects have shown repeatedly that there is often considerable additive genetic variation for resistance to parasites and pathogens (for a summary of selection experiments involving entomopathogenic viruses, see Fuxa and Richter, 1998). For example, laboratory experiments using colonies of velvetbean caterpillars, *Anticarsia gemmatalis*, collected from the USA and Brazil showed that following intense artificial selection for increased resistance to its nucleopolyhedrovirus (AgNPV) over four and 13 generations, respectively, resistance levels had increased by 5× and >1000× relative to insects in respective control colonies (Abot *et al.*, 1996). Two lines of evidence from subsequent studies on the US population suggest that virus resistance was costly. First, when artificial selection was discontinued, the resistant insects returned to their original level of susceptibility within just three generations (Fuxa and Richter, 1998). Moreover, two additional cycles of resistance selection and reversion were repeated in the same insect population with similar results, except that the responses were even quicker. This indicates that resistance was costly and that resistant genotypes were rapidly replaced by susceptible ones in the absence of the pathogen. Second, when insects from the resistant colony were compared with those from the susceptible colony, the former exhibited reduced fitness. Resistant females produced significantly fewer eggs and these had a significantly lower hatch rates, resulting in 61% fewer viable offspring being produced by resistant females than by susceptible ones. Resistant insects also had shorter lifespans, a lower rate of larval survival, and lower pupal weights than susceptible insects (Fuxa and Richter, 1998). Thus, the available evidence indicates that resistance to NPV in this moth population is 'bought' at the expense of costly reductions in reproduction and survival in the absence of the pathogen. However, these results must be treated with a certain degree of caution because the experiment was not replicated and so the robustness of the conclusions is unclear.

Two replicated artificial selection experiments using the fruitfly, *Drosophila melanogaster*, and its parasitoids provide convincing evidence for a cost of resistance. Four genetic lines were selected for resistance to attack by the braconid parasitoid wasp *Asobara tabida* and compared to four control lines that were not exposed to the parasitoid (Kraaijeveld and Godfray, 1997). Selected lines rapidly increased their cellular encapsulation response from 5%

at the start of the experiment to greater than 60% after five generations of selection (Fig. 10.5a). Initial investigations revealed little indication for a cost of resistance. However, after examining various life-history and other traits in the selected and control lines under a range of conditions, it became apparent that the main cost of resistance was a decline in the competitive ability of larvae in the selected lines relative to controls when food was in short supply (Fig, 10.5b). Similar experiments with the eucoilid wasp, *Leptopilina boulardi*, showed a similarly rapid response to selection, from less than 1% encapsulation at the start of selection to 45% after just five generations (Fellowes *et al*., 1998a). Significantly, increased resistance to parasitism by *L. boulardi* was also achieved at the expense of competitive ability when food was in limited supply. Subsequent studies indicated that this was because larvae from the selected lines had a lower feeding rate, an important determinant of larval competitive ability (Fellowes *et al*., 1999a). Larvae in the lines selected for resistance to *A. tabida* also had approximately twice the number of circulating haemocytes as those in the control lines (Kraaijeveld *et al*., 2001; Fig. 10.5c), and so it seems likely that their enhanced resistance was because they were better able to encapsulate the parasitoid larvae. This interpretation is consistent with the positive correlation observed across *Drosophila* species between encapsulation ability and both haemocyte counts (Eslin and Prévost, 1996, 1998; Fig. 10.5d), and metabolic rate (Fellowes and Godfray, 2000).

Thus, it appears that in the *D. melanogaster–A. tabida* system, there is a trade-off between feeding rate/competitive ability and haemocyte number/ encapsulation response. Why there should be a negative correlation between haemocyte count and feeding rate is unclear at present. One possibility is that there is a switch in the general energy budget of the larvae away from investment in trophic function to investment in the immune system. Alternatively, since both the head musculature and the haemopoietic organ (where the haemocytes are produced) both originate from the same part of the embryo, perhaps increased allocation of tissue to the future haemopoietic organ is at the expense of future muscle tissue. A third possibility is that a doubling of the number of circulating haemocytes increases the viscosity of the haemolymph, leading to lower rates of resource supply (e.g. glucose) to the muscles (Kraaijeveld *et al*., 2001, 2002).

A reverse approach to looking at the evolutionary costs of immunity was taken by Koella and Boëte (2002), who selected six lines of the mosquito, *Aedes aegypti*, for either early or late pupation and measured the extent to which this selection procedure changed the mosquito's ability to encapsulate and melanize a Sephadex bead. They found that after ten generations of selection, the age at pupation in the two selection regimes differed by about 0.7 days, showing that the selection procedure had worked. More significantly, they also found that whereas 32% of individuals from the lines selected for late pupation melanized the Sephadex bead, only 6% of mosquitoes from the lines selected for early pupation did so. Thus, there appears to be a genetic trade-off between the rate of larval development and the efficacy of the melanization response.

A third approach to examining the evolutionary costs of resistance has been to employ breeding studies (i.e. sib-analyses, offspring–parent regressions and
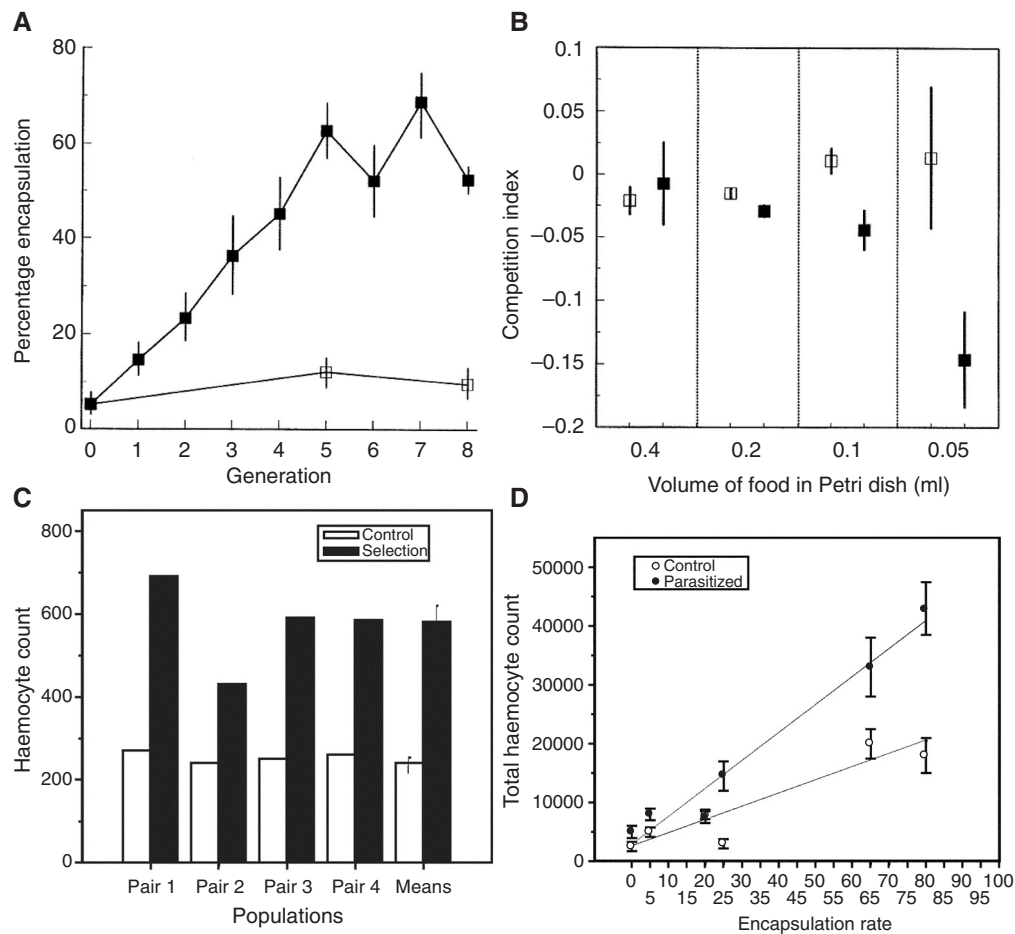
Fig. 10.5. Resistance to the braconid larval parasitoid *Asobara tabida* in the fruitfly *Drosophila melanogaster*. **A** The frequency of encapsulation in control lines of fruitflies and lines selected for resistance to *A. tabida*, showing means and standard errors of the four selected (*filled symbols*) and control lines (*open symbols*). **B** The competitive ability of experimental flies relative to a tester strain, showing means and standard errors of the four selected or control lines at four levels of competition for larval food. **C** Haemocyte counts in the four pairs of selected and control lines plus the overall mean (±SE) for both sets of lines. **D** Relationship between the encapsulation rate (ER) and the total haemocyte count (THC) recorded in both parasitized larvae (*closed symbols*), and control larvae (*open symbols*). Mean values (±SE) are given for six *Drosophila* species of the *melanogaster* subgroup: *D. sechellia* (*D. sech.*), *D. melanogaster* (*D. mel.*), *D. mauritiana* (*D. maur.*), *D. yakuba* (*D. yak.*), *D. teissieri* (*D. teis.*) and *D. simulans* (*D. sim.*). Figures **A** and **B** reprinted from Kraaijeveld and Godfray (1997); **C** reprinted from Kraaijeveld *et al.* (2001), and **D** from Eslin and Prévost (1998).

pedigree analyses; Falconer and Mackay, 1996; Roff, 1997). For example, Ryder and Siva-Jothy (2001) applied a sib-analysis approach to house crickets (*Acheta domesticus*) to examine the genetic correlations between two immune function traits (haemocyte load and encapsulation response) and a sexually-selected trait (male body size, which is strongly correlated with calling rate and the ability of a male to attract a mate). They found that all three traits were heritable and positively genetically correlated. Thus, females choosing large males with particular call characteristics will not only tend to produce larger offspring, but those offspring will have a greater ability to produce an encapsulation response. The costs associated with genetically large body size and encapsulation response have yet to be established. Recently, Cotter *et al.* (2004b) have applied a similar approach to the Egyptian leafworm (*Spodoptera littoralis*) to examine the genetic correlations between a suite of immune function traits (total haemocyte count, haemolymph phenoloxidase activity, lysozyme-like antibacterial activity, encapsulation response and degree of cuticular melanization) and a number of life-history traits (larval and pupal development rate, pupal weight, and adult longevity). They found that there was a complex mixture of positive and negative genetic correlations between immune function traits and life-history traits. But, most interestingly perhaps, they also observed a potential genetic trade-off within the immune system itself, with lysozyme-like antibacterial activity exhibiting negative genetic correlations with haemocyte density, haemolymph phenoloxidase activity and cuticular melanization. This result is consistent with those from other studies showing negative *phenotypic* correlations between antibacterial activity and either phenoloxidase activity (in the bumblebee, *Bombus terrestris*; Moret and Schmid-Hempel, 2001) or encapsulation response (in the Mediterranean field cricket, *Gryllus bimaculatus*; Rantala and Kortet, 2003). Although conflicts between responses tailored to specific parasites have been predicted (Hamilton and Zuk, 1982), this is perhaps less surprising than the possibility of a trade-off between general components of the innate immune system. Further studies are clearly required before a trade-off between the humoral and cellular arms of the insect immune system can be verified, but it is worth noting that there is some evidence for these sorts of negative genetic correlations from vertebrate studies (e.g. Gross *et al.*, 1980; Grencis, 1997; Gehad *et al.*, 1999; Ibanez *et al.*, 1999; Johnsen and Zuk, 1999; Gill *et al.*, 2000).

A likely consequence of negative genetic correlations between different components of the immune system is that we can also expect negative correlations between levels of resistance to different parasite types. So far, few studies have examined this possibility. Fellowes *et al.* (1999b) found no evidence for the existence of trade-offs between resistance to different parasitoid species in *D. melanogaster*. In fact, selection for increased resistance to *A. tabida* and *L. boulardi* both resulted in concomitant increases in resistance to a third parasitoid, *Leptopolina heterotoma*, suggesting that in both sets of selection lines some attribute of general utility in resisting parasitoids was being selected for. However, some more specific attributes were also selected for in the lines resistant to *L. boulardi*. This is indicated by the fact that although selection for increased resistance to *L. boulardi* led to levels of resistance against *A. tabida*

that were similar to those in the *A. tabida* selection lines, selection for increased resistance to *A. tabida* did not result in increased levels of resistance to the more specialized parasitoid, *L. boulardi* (which produces immunodepressive virus-like particles, unlike *A. tabida*, which evades the *Drosophila* immune system by hiding within host tissues). It remains to be established whether there are trade-offs between resistance to parasitoids and resistance to more distantly related parasites (e.g. bacteria, fungi, etc) that may be combated by different components of the immune system.

Understanding the costs of resistance potentially has a number of applied ramifications. For example, there are hopes that it will one day be possible to genetically manipulate the immune response of *Anopheles* mosquitoes in order to make them refractory towards *Plasmodium* parasites, and so control the spread of malaria (Collins, 1994; Collins and Paskewitz, 1995). These hopes have been raised recently by the observation that the immune response has a genetic basis (e.g. Collins *et al.*, 1986; Dimopoulos *et al.*, 2002; Thomasova *et al.*, 2002), and genes involved in the immune response are being located and mapped in the mosquito's genome (e.g. Gorman *et al.*, 1997). However, the efficacy of such an approach may depend on inherent genetic trade-offs, as well as on the extent to which the insect's immune response depends on non-genetic factors, such as the host's diet, age, sex and reproductive status (Koella and Sorensen, 2002).

4. Case studies

In this section, we apply the ecological immunology approach to examine three areas of interest to evolutionary ecologists: (a) density-dependent prophylaxis; (b) group-living and disease risk; and (c) sex-biased parasitism. Each of these examples comes from our own work, but hopefully illustrate general principles that apply not only to insects, but also to other animal groups, including vertebrates.

4.1 Density-dependent prophylaxis

Most pest species of insects, almost by definition, exhibit wide fluctuations in population density from one generation to the next: when conditions are favourable, population densities are high, and when they are less favourable they are low. For example, population densities of the larch budmoth (*Zeiraphera diniana*), a lepidopteran forest pest, may vary by more than 20,000-fold over five generations (Speight *et al.*, 1999). Low- and high-density environments differ in a number of qualitative and quantitative ways. Most obviously, of course, the levels of competition for food will vary, and this may impact on the insect's capacity to 'feed' its immune system (see above). Another important aspect of the environment that differs between low- and high-density populations is the degree of exposure to parasites and pathogens (Wilson and Reeson, 1998). This is because most pathogens tend to be transmitted in a positively density-dependent manner (Anderson and May, 1979; McCallum *et*

al., 2001) – as population density increases, so the probability of infectious and susceptible hosts coming into contact increases. As a consequence, insects in crowded populations will generally experience greater risk of exposure to pathogens than those in low-density populations (though the relationship between infection risk and population density may not be linear; *e*.g. Hochberg, 1991a; McCallum *et al*., 2001). Thus, we can expect animals to tailor their investment in disease resistance mechanisms to match the perceived risk of infection, using population density as a cue to their infection risk.

This 'density-dependent prophylaxis (DDP) hypothesis' (Wilson and Reeson, 1998) rests on two important assumptions. The first is that investment in prophylactic resistance mechanisms is costly – if resistance was cost-free, then these resistance mechanisms would always be expressed. As we saw in the previous section, there are good theoretical grounds for assuming that resistance will be costly, though the exact costs of maintaining prophylactic resistance are not well characterized (see above). The second assumption is that insects have reliable mechanisms for perceiving local population density and adjusting their phenotype accordingly. It has long been established that such phenotypic plasticity may be manifested in insects, and the phenomenon has been termed 'density-dependent phase polyphenism'. It is typified by the desert locust, *Schistocerca gregaria*, which, in the nymphal stages, exhibits a green, cryptic phenotype adapted to low-density conditions, and a conspicuous yellow-and-black phenotype adapted to high-density conditions. Switching between these two phenotypes is largely determined in response to tactile cues, especially those perceived by the hind-femurs, during nymphal development (though visual and olfactory cues are also important; Simpson *et al*., 2001).

Given that at least some insects are capable of adjusting their phenotype in response to cues reflecting local population density (and infection risk), and that maintaining disease resistance mechanisms exact some cost, is there any evidence that insects alter investment in disease resistance mechanisms to reflect their perceived risk of infection? A number of studies over the past few years have provided evidence in support of this notion. The first direct test of the DDP hypothesis was conducted using the African armyworm, *Spodoptera exempta* (Reeson *et al*., 1998, 2000). The larval stage of this moth is an economically important pest of pasture grasses and graminaceous crops in eastern Africa, and densities of this 2–3-cm-long caterpillar can reach anything up to 1000 per m^2, and occasionally more (Rose *et al*., 2000). Population densities vary considerably between years: in Tanzanian light traps, a survey conducted over a 30-year period indicated that the number of moths caught varied between just 150 to more than 300,000; a 2000-fold difference (Harvey and Mallya, 1995). Moreover, there is little correlation between the numbers of moths caught in successive years. For example, in 1976 more than 60,000 moths were caught in Tanzanian light traps, compared with just 800 moths in the following year. In response to this unpredictable variation in population density, just like the desert locust, *S. exempta* has evolved density-dependent phase polyphenism: at low population densities, the larvae are usually green or pale brown, whereas at high densities, they are invariably jet black, due to the deposition of melanin in the cuticle (see Fig. 10.3; Wilson *et al*., 2001).

To determine whether disease resistance was related to larval phenotype, Reeson *et al*. (1998) reared *S. exempta* larvae under either high- or low-density conditions in the laboratory and then orally challenged early fourth-instar larvae with one of five known doses of *S. exempta* NPV via the diet-plug method. This baculovirus is probably the main pathogen impacting on the fitness of African armyworms and has been known to cause greater than 90% mortality in some larval outbreaks (Rose *et al*., 2000). Its main mode of transmission is probably horizontal, via the ingestion of virus-contaminated vegetation (but vertical transmission is also known to occur; see Swaine, 1966), and so the *per capita* risk of infection is likely to increase in a positive density-dependent manner.

In this experiment, approximately 15% of larvae reared under low-density conditions expressed a phenotype that was more like that of larvae reared under high-density conditions (reflecting genetic variation in the larval density required to trigger the switch into the high-density phenotype). Thus, Reeson and colleagues determined the LD_{50}s for the typical green-brown low-density pheno-type, the typical melanic high-density phenotype, and the atypical melanic phenotype produced under low-density conditions (which, in fact, is less melanic than the typical crowded phenotype; Wilson *et al*., 2001). They found that, as predicted, the LD_{50} increased as the larval phenotype switched from the typical solitary to the typical crowded forms (Fig. 10.6a). In fact, approximately ten times more viral occlusion bodies are required to kill a typical crowded larva as a typical solitary one (Reeson *et al*., 1998). Moreover, similar results were gained when the viral infection was gained via natural exposure to the virus in a field situation (Reeson *et al*., 2000). Significantly, this difference in viral susceptibility was mirrored by similar variation in constituitive levels of phenoloxidase activity (Fig. 10.6b), suggesting a possible involvement of this enzyme cascade in resistance to baculoviruses (see also Washburn *et al*., 1996).

Similar experiments on other Lepidoptera (Mitsui and Kunimi, 1988; Kunimi and Yamada, 1990; Goulson and Cory, 1995; Wilson *et al*., 2001; Cotter *et al*., 2004a), tenebrionid beetles (Barnes and Siva-Jothy, 2000) and the archetypal phase polyphenic species, the desert locust (Wilson *et al*., 2002), generally show similar density-dependent increases in disease resistance and/or immune function. Future studies on this subject need to identify the precise costs associated with the density-dependent increase in immune function. Attempts to do this so far have generally proved unsatisfactory (see above), due mainly to the fact that a whole suite of coordinated immunological and non-immunological traits are simultaneously altered in relation to population density, and so dissecting the relative costs and benefits of any particular phenotypic change is difficult. This is particularly so because there will also be compensatory changes in diet and metabolism to ameliorate any costs. The problem associated with identifying the costs of density-dependent prophylaxis is analogous to that associated with quantifying the costs of migration (Dingle, 1991, 1996; Wilson, 1995). The emergence of 'migration syndromes' (Dingle, 1991, 1996) to minimize the costs of migration has thwarted efforts to accurately quantify the costs involved. Ecological immunology might benefit from the lessons learned by migration biologists.
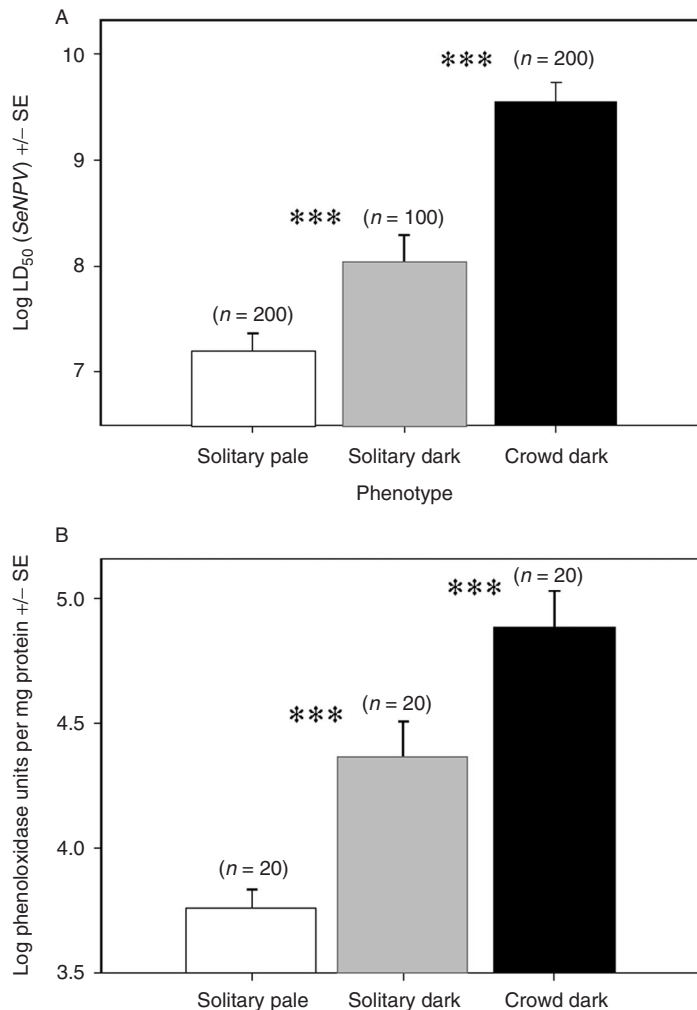
Fig. 10.6. Relationship between *Spodoptera exempta* larval phenotype and **A** resistance to S. exempta nucleopolyhedrovirus and **B** phenoloxidase (PO) activity. In **A**, resistance is measured via mean LD_{50} (+SE.) determined using five different doses of the baculovirus (redrawn after Reeson *et al.*, 1998). In **B**, mean PO activity (±SE) is expressed as PO units per milligram protein (redrawn after Wilson *et al.*, 2001). The three larval phenotypes are typical (non-melanic) larvae produced under solitary rearing conditions, typical (melanic) larvae produced under crowded rearing conditions, and (atypical) melanic larvae produced under solitary rearing conditions (see Fig. 10.3).

Future studies also need to establish the speed and specificity of density-dependent changes in prophylactic immune function. Desert locust nymphs start exhibiting gregarious behaviour within just 4 h of receiving tactile stimuli characteristic of high-density, crowded conditions (Simpson *et al.*, 2001). This leads to the obvious questions of how quickly prophylactic disease resistance mechanisms can be upregulated in response to the appropriate stimuli; whether

downregulation subsequently occurs in the absence of such cues; and whether the cues used for immunological transformation are the same as those used for behavioural transformation.

Although we have focused on density-dependent changes in investment in prophylactic immune function, the same principle applies to any cue that reliably predicts infection risk. For example, if there are reliable seasonal changes in infection risk, then we might expect insects to use seasonal cues (such as daylength, temperature and humidity) when making decisions about relative investment in immune function. Whilst we are aware of no insect examples of this phenomenon, it has been hypothesized that adaptive seasonal variation in immune function occurs in rodents (Nelson *et al.*, 1996a,b, 1998). Similarly, geographical or climatic variation in prophylactic immune function might be expected to evolve under some scenarios.

Recently, Moret and Siva-Jothy (2003) have added another example to this list. They argue that one of the best cues predicting future exposure to parasites is previous exposure to them and, therefore, we can expect 'responsive-mode prophylaxis' to evolve. They tested this idea using larvae of the mealworm beetle, *Tenebrio molitor*. They found that when larvae were pre-challenged with LPS, they produced antibacterial responses that lasted at least 7 days. Moreover, relative to control insects, the LPS-treated larvae exhibited greater resistance to infection by the entomopathogenic fungus, *Metarhizium anisopliae*, when they were exposed 4 or 7 days after the pre-challenge. Thus, Moret and Siva-Jothy argue that the long-lasting antimicrobial responses of invertebrates may serve a similar function to the adaptive immune responses of vertebrates in providing better protection against repeated parasitic infections, though the specific memory of these prophylactic responses is clearly not comparable (see above). An earlier study of the dampwood termite, *Zootermopsis angusticollis*, also found that prior exposure to parasite antigens can offer enhanced protection against parasites in insects. Rosengaus *et al.* (1999b) found that nymphs immunized with an injection of glutaraldehyde-killed bacteria (*Pseudomonas aeruginosa*) had significantly higher survival than controls following a challenge with a lethal concentration of live bacteria. Similarly, nymphs exposed to a low dose of an entomopathogenic fungus (*Metarhizium anisopliae*) survived better than control insects after a challenge with a lethal concentration of live spores. Thus, prior exposure to a pathogen conferred upon termites a degree of protection during a subsequent encounter with the same pathogen. Unfortunately, Rosengaus and colleagues did not determine whether exposure to dead *P. aeruginosa* afforded protection to *M. anisopliae*, and so it is not possible to determine the specificity of this response.

4.2 Group-living and disease risk

An intuitive extrapolation of the DDP hypothesis outlined above is that, across species, we might expect group-living insects to invest more in prophylactic disease resistance than solitary-living insects. This is because group-living insects will typically experience higher local densities than solitary-living ones

and hence, presumably, higher *per capita* infection risk. Increased parasitism as a cost of group-living has long been assumed, but the evidence for it is equivocal at best (Freeland, 1979; Davies *et al*., 1991; Coté and Poulin, 1995). The idea was first tested in insects by Hochberg (1991b), who used data extracted 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 the idea that gregariously feeding insects are investing more in disease resistance mechanisms, this analysis was potentially confounded by host and pathogen phylogenies, as well as several other variables such as host diet, rearing conditions and coevolved resistance mechanisms.

To test this idea further, Wilson *et al*. (2003a) took a different approach to the problem. Instead of measuring pathogen resistance directly, they measured several aspects of immune function. To minimize the influence of potentially confounding variables, they assayed immune function in 12 lepidopteran species arranged in six phylogenetically matched pairs of species, assayed at similar times and raised on the same diet and in the same environmental conditions. They found that, although there was considerable variation across species in the density of haemocytes in the haemolymph (i.e. total haemocyte count; THC) across the six species-pairs, the THC of the solitary species was, on average, 40% higher than that of the gregarious species. Moreover, all six solitary species had THC values that were higher than their paired gregarious species (Fig, 10.7a). Similar results were apparent in the phenoloxidase activity, except that levels were higher in the solitary species in only five of the six species-pairs. Thus, contrary to the initial expectation, it appears that solitary species are investing more in immune function than gregarious species.

There are at least two possible explanations for this counterintuitive result. The first is that high densities of haemocytes in the haemolymph and high phenoloxidase activity levels do not, in fact, reflect differences in disease resistance. However, there is good evidence from a number of studies that high phenoloxidase activity enhances parasite resistance (see above), and that high THC enhances both the capacity to phagocytose novel antigens (e.g. Kurtz, 2002) and the capacity to encapsulate at least some parasites (Eslin and Prévost, 1996, 1998; Kraaijeveld *et al*., 2001; Rantala *et al*., 2000; Fig. 10.5d). Moreover, Wilson and colleagues showed that, for the species in their analyses, there was a strong positive correlation between THC (and PO) and the magnitude of the encapsulation response (Fig. 10.7b). This suggests that the difference in THC and PO related to larval feeding habit genuinely reflects investment in immune function.

A second possible explanation for the observed results is that the relationship between group-living and infection risk is not as previously stated. To investigate this possibility further, Wilson *et al*. (2003a) 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. In the model, there was a regular network of sites, each taking one of three possible states: empty, occupied by a
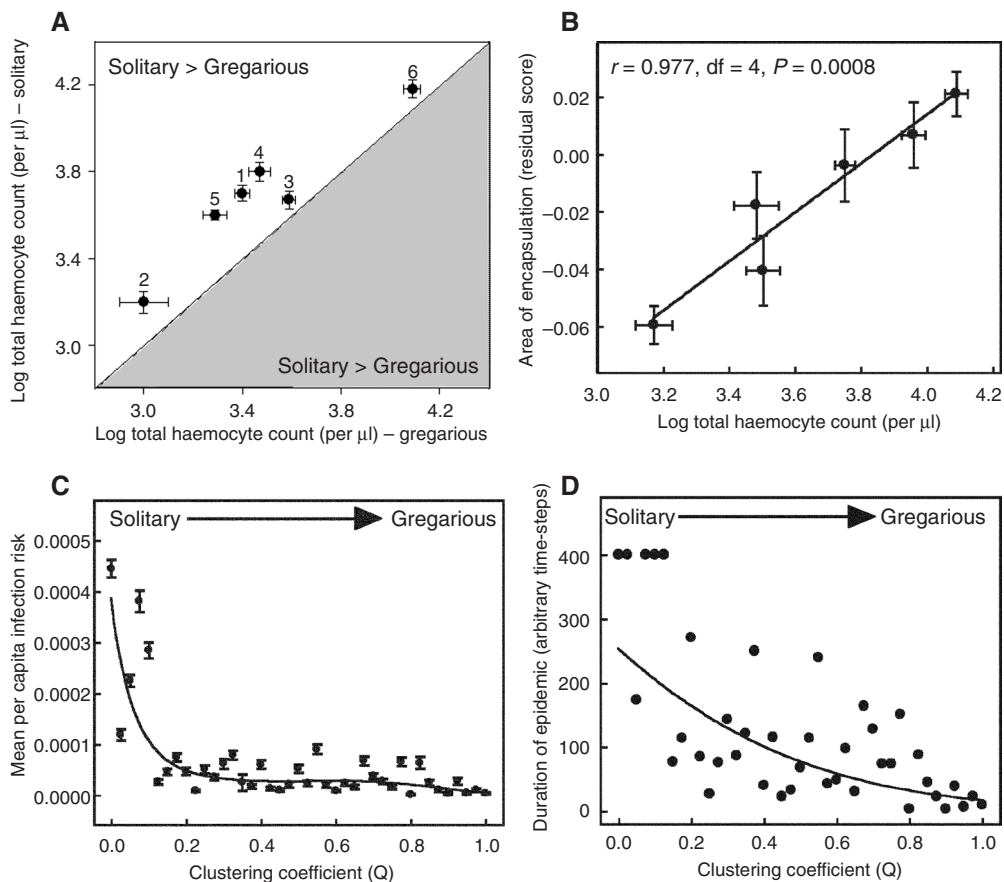
Fig. 10.7. Group-living and risk of disease in lepidopteran larvae. **A** Relationship between feeding style and total haemocyte count (THC); gregariously-feeding species have significantly lower THC than solitary-feeding species (means ± SE shown). **B** Relationship between THC and magnitude of encapsulation response directed against a nylon implant by six species of lepidopteran larvae (means ± SE shown). Relationship between the degree of host-clustering and **C** mean *per capita* infection risk (±SE) and **D** duration of epidemic. Data are output from a dynamic, susceptible/infected spatially-explicit model. Reprinted from Wilson *et al.* (2003a).

susceptible host, or occupied by an infected host (*see* Boots and Sasaki, 2000, for a detailed explanation of this modelling approach). Infection occurs when there is contact between neighbouring infected and susceptible individuals. A site becomes empty when an individual dies, and it is then available to be re-occupied by the progeny of other individuals. The key parameter in this model, in terms of the present discussion, is the clustering coefficient, *Q*, which determines the proportion of offspring that are born into neighbouring sites, as opposed to randomly across the whole lattice. Changes in this clustering coefficient therefore produce populations with different average local clustering.

Using this model, Wilson and colleagues showed that, for a significant proportion of parameter space, host clustering can reduce individual infection risk in both endemic and epidemic host–pathogen interactions. Thus, in the epidemic scenario, in which a disease epidemic spreads through the population, as the clustering coefficient increases (and individuals become increasingly aggregated in groups), so the mean *per capita* risk of becoming infected declines, as does the duration of the epidemic (Fig. 10.7c, d).

So, it appears that aggregation in clusters might actually reduce the probability of becoming infected by a disease agent. But how does this happen? The mechanism behind this phenomenon is fairly simple. 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. Therefore, the 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 (Wilson *et al*., 2003a). Similar conclusions were drawn from a simpler epidemiological model by Watve and Jog (1997). Of course, group-living will fail to be advantageous in this context when the parasite is highly mobile (or transmitted by a mobile vector) and so not constrained by the spatial distribution of its host, or when hosts are highly mobile or at such low densities that the infection risk is low for all hosts.

Thus, both the models and the immune function assays suggest that group-living may lead to reduced, rather than increased, risk of becoming infected by parasites under some circumstances. What are needed now are replicated experiments in which insect group size is manipulated and the spread of an introduced pathogen is monitored. Only then can we be sure whether group-living enhances or reduces disease risk in Lepidoptera–pathogen interactions. Regardless of the results of such experiments, the take-home message from this study is that, in combination with other relevant methods, ecological immunology studies can both challenge and generate novel predictions regarding the evolutionary ecology of insect host–pathogen interactions.

It seems likely that the relationship between sociality and disease risk will depend critically on the interaction between the insect's social system and the nature of the pathogenic challenge (Wilson *et al*., 2003a). For example, it has recently been suggested that 'social transfer' of infection resistance might yield a survivorship bonus in social insects (Traniello *et al*., 2002). In the termite, *Zootermopsis angusticollis*, survival following infection with the entomopathogenic fungus, *M. anisopliae*, was significantly enhanced if infected individuals were subsequently reared in groups rather than in isolation. Moreover, termite survival following exposure to a lethal dose of fungal spores was significantly enhanced if naïve individuals were allowed to associate with previously immunized nestmates before they were infected (Traniello *et al*., 2002). These 'socially immunized' termites were approximately 34% less susceptible to a lethal infection than termites that were not allowed contact with immunized siblings. Yet again, the precise

mechanisms underpinning these patterns remain to be established, but other mechanisms used by termites to minimize infection risk include mutual grooming, scaled in frequency to pathogen prevalence (Rosengaus *et al*., 1998b, 2000b; see above); the production of antibiotic secretions in exocrine glands and other exudates (Rosengaus *et al*., 1998a, 2000a); and the communication of information about the presence of pathogens in the nest (Rosengaus *et al*., 1999a).

4.3 Sex-biased parasitism

Male and female life histories are usually very different, especially in species with highly polygynous mating systems, in which females maximize fitness by living for a long time and producing many young, and males maximize fitness by mating with many reproductively active females (Bateman, 1948; Trivers, 1972). These disparate selection pressures often lead to the production of weapons and ornaments in males, as well as large body size, especially in mammals (Andersson, 1994). These traits are envisaged to be costly to produce and maintain and, in mammals, it has been shown that in species in which there is strong sexual selection (as measured by mating system and sexual size dimorphism), males appear to suffer a viability costs (i.e. there is male-biased mortality). Recently, it has been suggested that one mechanism by which this viability cost might be exerted is via increased susceptibility or exposure to parasites (Moore and Wilson, 2002). Indeed, across a range of mammal species, males suffer significantly greater prevalence of parasitism (via a range or parasites and pathogens) than do females, and this sex-bias in parasitism (SBP) is positively correlated with the strength of sexual selection: SBP is greater in polygynous than monogamous species, and is positively correlated with the extent of sexual size dimorphism (Moore and Wilson, 2002), but not with sex differences in home-range size (Wilson *et al*., 2003b). Moreover, there was a positive correlation between SBP and sex-biased mortality, with species in which there was male-biased parasitism also exhibiting male-biased mortality. These analyses are consistent with the idea that sexual selection imposes a viability cost on males and that this cost is mediated, in part at least, by sex differences in parasitism, although the precise mechanism causing differential parasitism of the sexes remains to be identified (Moore and Wilson, 2002).

If sex-biased parasitism is a general cost of sexual selection, then we should also observe SBP in other taxonomic groups. Sex differences in parasitism have been observed in humans (e.g. Owens, 2002; Wilson *et al*., 2003b) and birds (Poulin, 1996), but were not found in fish (Poulin, 1996) or in invertebrates, including insects (Sheridan *et al*., 2000). However, a recent study of insects, using a much larger dataset than that used by Sheridan and colleagues, observed a small, but highly significant, male-bias in the prevalence of parasitism by a range of entomopathogens (S.L. Moore and K. Wilson, unpublished). Moreover, as with the mammal analysis, the male-bias was statistically significant for polygynous species, but not for non-polygynous (monogamous and polyandrous) ones, and was significantly positively correlated with the degree of sexual size dimorphism.

Interestingly, a meta-analysis of published information on sex differences in traits associated with immune function in insects, namely haemocyte number, antibacterial activity and phenoloxidase activity, indicated that females have consistently higher levels than males (S.L. Moore and K. Wilson, unpublished). Thus, sex differences in immune function may explain why males are generally more heavily parasitized than females, though clearly more detailed species-specific studies are required. This result is particularly interesting because it shows that in both vertebrates and invertebrates females have better immunity and lower parasite loads than males, suggesting a common explanation. This is interesting because it suggests that we can probably dismiss the immuno-depressive effects of testosterone as an important explanation for sex differences in parasitism in vertebrates (Folstad and Karter, 1992), since we get the same pattern in insects which, of course, lack testosterone or any sex-specific hormones (Nijhout, 1994). However, recent studies have shown that mating may have immunodepressive side effects in insects (e.g. McKean and Nunney, 2001; Rolff and Siva-Jothy, 2002), and that these effects may be mediated by juvenile hormone (Rolff and Siva-Jothy, 2002). So, if males mate more frequently, on average, than females, then they may spend relatively more time in an immunodepressed state and this might explain their greater parasitism.

5. Concluding remarks

These three case studies illustrate the sort of approach taken by ecological immunologists to understand the evolutionary ecology of insect host–parasite interactions. They show that there are consistent and predictable differences between individuals, sexes and species in terms of their investment strategies in parasite defence mechanisms, and that these can be understood by considering details of the ecology and evolutionary background of the species concerned. For example, within species, individuals respond to the perceived risk of parasitism by investing in mechanisms that reduce their susceptibility to pathogens. Across species, group-living insects tend to exhibit reduced haemocyte production and reduced phenoloxidase activity, suggesting that they may experience a reduced risk of infection from at least some types of parasite relative to solitary-feeding species. And finally, within species, males tend to have weaker immune responses than females and, as a result, tend to have a higher prevalence of parasitism. The prospects for ecological immunology making further significant basic and applied contributions to the study of insect host–parasite interactions looks promising.

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