PLASTICITY IN IMMUNE RESPONSES IN A MODEL INSECT

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Abstract

In order to understand how the environment, parental environment and inbreeding influence immune function and life history traits, I carried out a series of experiments using the Indian meal moth, *Plodia interpunctella*, as a model organism.

Past studies have focused mainly on one aspect of environmental fluctuation at a time, so, to study the nature of the interactions between environmental variables in determining immunity, temperature, food quality and density were all varied together. There were interaction effects on immunity between all three environmental variables. The effect of density, for example, can be reversed if diet is changed from high- to low-quality.

Diet quality has been shown to affect many life-history traits and an interesting question arising from this is whether these effects carry on to the next generation. I showed that the negative effects of a poor diet can be seen in the offspring of those affected but that a good quality diet given to the offspring goes some way to ameliorate these effects. Some of these effects on offspring could be mediated by maternal investment in their eggs; for example, when females were mated to a male that had received a poor quality diet, egg sized showed a strong effect of maternal size, with smaller females laying smaller eggs.

Finally, I addressed the question of how inbreeding affects immune function using *P. interpunctella* derived from Australian stocks as well as a UK culture and I showed that there are some high costs to be paid in terms of larval size, egg size, larval survival, protein content of the haemolymph and immune function. There were also strong effects of origin, for example Australian males showed a very marked reduction in PO activity with inbreeding, but Australian females and both sexes from the UK lines did not show a great reduction with inbreeding.

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CHAPTER 1: Introduction

Invertebrates are frequently exposed to pathogens and parasites throughout their lives. Therefore, selection will exert pressure on them to evolve and maintain an immune system for defence (Kraaijeveld et al. 2002). For a response to be generated, a pathogen has to be recognised as 'non-self'. Insects lack immunoglobulins, the highly specialised gamma globulin proteins vertebrates use to identify and 'remember' an intruder, yet they continue to thrive in a pathogen-laden world due to an effective innate immunity. This consists of general responses such as anti-microbial peptides, phagocytosis, pattern-recognising receptors such as those that recognise bacterial lipopolysaccarides (LPS), the phenoloxidase cascade and melanisation reaction and interference of virus RNA (Loker et al. 2004). Furthermore, there is the possibility of many other responses that we are not aware of; for example, over 200 genes are switched on after *Drosophila* are exposed to a pathogen, but for many of them the function is unknown (De Gregorio et al. 2001). Invertebrates and vertebrates do, however, share some similarities that allow invertebrates to be valuable model species in the study of how innate immunity in vertebrates may work (Medzhitov et al. 1997; Schnare et al. 2001; Hoffmann and Reichhart 2002; Takeda et al. 2002; Takeda and Akira 2003; Takeda et al. 2003).

Innate immunity is a general response to infection, whereas acquired immunity is a result of the production of molecules that respond to specific antigens (Bendelac and Fearon 1997; Fearon 1997b, a). Insects have a very well-developed innate immune system that can be subdivided into humoral immune responses, such as antibacterial

proteins and enzyme cascades (Bulet 1999; Bulet et al. 1999; Blandin and Levashina 2004; Bulet and Stocklin 2005) and cellular immune responses, such as phagocytosis and encapsulation (Strand and Pech 1995; Muta and Iwanaga 1996; Gillespie et al. 1997; Irving et al. 2005). The categories of humoral and cellular immunity are used to allow for ease of discussion, but in nature the lines between them are blurred; for example, humoral molecules coordinate haemocyte activity, and haemocytes produce many humoral molecules (Strand 2008).

1.1 The cellular immune response

Haemocytes are cells circulating in the haemolymph of invertebrates. The density of these cells is known to correlate with an individual's ability to encapsulate a wound site or intruder, as well as resist bacterial and viral attack (Eslin and Prévost 1996; Wilson et al. 2003a). They are responsible for the phagocytocis of intruding cells and they aggregate around a wound site or large intruder before being melanised to form a protective shell or capsule (Soderhall and Smith 1983; Ratcliffe et al. 1984; Leonard et al. 1985; Soderhall et al. 1986).

Haemocytes can be divided into categories based on their morphology. Plasmatocytes are the most abundant type and form the bulk of the capsule around a foreign intruder and phagocytose foreign material (Ratcliffe and Gagen 1976; Lavine and Strand 2002; Asha et al. 2003). Granular cells are mainly responsible for phagocytosis (Mazet et al. 1994; Yokoo et al. 1995; Pendland and Boucias 1996; Ribeiro et al. 1996; Tojo et al. 2000; Costa et al. 2005) but help remove bacterial proteins from the haemolymph and

release their granules once in contact with a larger foreign body (Ratcliffe and Gagen 1977; Schmit and Ratcliffe 1977; Ratcliffe and Rowley 1979). Lamellocytes are not found in healthy individuals but are rapidly produced upon attack by parasitoids and during metamorphosis (Lanot et al. 2001; Meister and Lagueux 2003) and function to encapsulate large foreign objects. Oenocytoids are non-adhesive fragile cells that release the enzyme phenoloxidase, important in the melanisation of the protective capsule around a foreign body or wound site, into the haemolymph when they lyse (Ashida and Yoshida 1988; Ribeiro et al. 1996). Spherule cells are large irregular cells whose function is unknown but they possibly contain cuticular proteins (Lavine and Strand 2002; Ribeiro and Brehelin 2006). There are also small haemocytes circulating that function as prohaemocytes, ready to differentiate into a terminal type of haemocyte when needed (Lanot et al. 2001; Strand 2008).

Phagocytosis

Phagocytosis is a type of endocytosis triggered when specialised receptors detect a foreign particle (Ehlers et al. 1992; Rohloff et al. 1994) such as bacteria, yeast, apoptotic bodies and a number of manmade items such as synthetic beads and India ink particles (Lanot et al. 2001; Lavine and Strand 2002).

Microbial molecules (Huxham and Lackie 1988) and the prophenoloxidase activating cascade (Johansson et al. 2000) may stimulate the haemocytes to increase their phagocytic rate. Granulocytes and plasmatocytes are the cells mainly responsible for phagocytosis in invertebrate immune systems (Ratcliffe and Rowley 1979; Wago 1991;

Tojo et al. 2000). However, they differ in phagocytosis rate in vivo and in vitro (Ehlers et al. 1992) and the methods by which the haemocyte kills the microbe once it has been engulfed are still unclear.

Nodule formation

In response to microbial proteins, haemocytes can clump together ensnaring a large number of bacteria in a mass, called a nodule, which may eventually become encapsulated (Lackie 1988). Proteins that may help bacterial adhesion within the nodule are found in *Ceratitis capitata* (the Mediterranean fruit fly) haemolymph after LPS or bacterial injection (Marmaras and Charalambidis 1992; Marmaras et al. 1994; Charalambidis et al. 1995; Kyriakides et al. 1995). In *Manduca sexta* (the tobacco hornworm) a sugar-binding protein called scolexin is released by the epidermis and midgut upon bacterial infection or wounding and is eventually found concentrated within nodules (Kyriakides et al. 1995).

Encapsulation, phenoloxidase and melanin

When a large foreign body that is unable to be phagocytosed is detected haemocytes will cluster around it forming a capsule that may eventually be melanised to form a protective capsule; this can stop the growth or spread of a pathogen and even kill it (Sugumaran 2002a). Encapsulation is a complex process generally started by granular cells, which then release compounds to attract plasmatocytes to join the capsule forming a thick shell around the foreign body (Davies and Siva-Jothy 1991; Pech and Strand 1996, 2000). In some species the haemocytes are arranged in a definite order in a

capsule but in others the arrangement is much more random (Wiegand et al. 2000).

Encapsulation is a common component of the cellular immune response in Lepidoptera, and the capsule is often, although not always, melanised by the phenoloxidase cascade (Soderhall 1982; Götz 1986; Binnington and Barrett 1988). In the Diptera, humoral melanotic encapsulation is more common (Götz 1986), as it can occur with or without haemocyte involvement (Christensen and Seversen 1993). Cellular encapsulation in Diptera is less clear (Christensen and Seversen 1993); however, it has been shown that lysis of haemocytes occurs before encapsulation (Russo et al. 1995; Hernandez-Martinez et al. 2002).

Phenoloxidase (PO) is a copper-dependent enzyme that catalyses phenoloxidation to produce, among other things, melanin from substrates such as dihydroxyphenylalanine (L-dopa). It exists in haemocytes as the inert prophenoloxidase, and it has been shown that bacterial lipopolysaccharides (LPS) can trigger the cascade to turn the inert precursor into the immune active PO (Soderhall 1982; Soderhall and Hall 1984). Haemocyte aggregation, phagocytosis and exocytosis are also triggered by this cascade (Schmit and Ratcliffe 1977; Ratcliffe et al. 1984; Leonard et al. 1985; Soderhall et al. 1986), while some intermediate compounds of the cascade react with proteins and amino acids to inhibit microbial & mycelial enzymes, denature proteins and lyse or kill foreign cells (Mason 1955; Pawelek and Lerner 1978; Soderhall and Ajaxon 1982; Johansson and Soderhall 1989). The deposition of melanin produces cytotoxic effects due to superoxide anions and hydroxyl radicals that may prove fatal to invading

microbes (Nappi et al. 1995a). Melanin also serves to harden the culticle, which can help prevent injury and infection (Hopkins and Kramer 1992; Sugumaran 1996; Sugumaran et al. 1996; Gillespie et al. 1997; Sugumaran 1998; Sugumaran 2002a). PO is extremely important and *Drosophila* with a null PO mutation have much reduced survival (Asada et al. 1999).

Wounding threatens insect survival as it can cause a large amount of haemolymph to be lost if the breach is not sealed quickly. After wounding, the PO cascade is rapidly triggered which causes melanin and quinoid products to build up in a cross-linked pattern to seal the gap (Ashida and Brey 1995; Sugumaran 1996). These quinoids produced by the PO cascade also act as a disinfectant, killing any invading organisms at the wound site (Nappi et al. 1995a; Sugumaran 2002a; Sugumaran 2002b).

1.2 The humoral immune response

Antibacterial Proteins

Many bacteria-induced proteins are found in insects. Lysozyme is constantly present and active in insects. In Lepidopterans it is secreted in response to bacterial infection where it hydrolyses glycosidic bonds in the cell walls of the invading bacteria; however, in *D. melanogaster* it serves as a digestive enzyme only (Gillespie et al. 1997). Cecropin peptides are cationic and are thought to lyse bacterial cells by interfering with cell membrane structure (Gazit et al. 1994). Attacin/sarcotoxin II proteins are bacteriostatic. They are induced by the presence of gram-negative bacteria and act only

on them to prevent production of bacterial outer membrane proteins, so halting cell division (Carlsson et al. 1991). Diptericins are similar to attacins but have been found only in dipterans (Dimarcq et al. 1986; Keppi et al. 1986; Dimarcq et al. 1988; Lambert et al. 1989; Reichhart et al. 1989; Wicker et al. 1990; Ishikawa et al. 1992). Defensins only act on gram-positive bacteria, forming channels in the cell membranes to lyse the cells (Cociancich et al. 1993). They have been found in several insect orders (Bulet et al. 1991; Hoffmann and Hetru 1992; Chalk et al. 1994; Dimarcq et al. 1994) and despite their name are not homologous to mammal defensins (Hetru et al. 1994). These antibacterial proteins and peptides are released into the haemolymph, mostly by the fat body but they can also be produced by haemocytes, pericardial cells, Malphigian tubules, the midgut and epidermal cells (Dickinson et al. 1988; Russell and Dunn 1991; Brey et al. 1993; Dunn et al. 1994; Mulnix and Dunn 1994; Lee and Brey 1995; Russell and Dunn 1996).

Antifungal Proteins

Pathogenic fungi are regularly responsible for insect death and some antifungal peptides have been found in insects. A histidine-rich protein was discovered in *Sarcophaga peregrina* (the Flesh-fly) that, when added to culture media, inhibits the growth of *Candida albicans* (Iijima et al. 1993), and similar proteins have also be purified from *Tenebrio molitor* (the Meal Worm Beetle) and *Holotricia diomphalia* (a scarab beetle) haemolymph (Jung et al. 1995; Lee et al. 1995). Other types of antifungal peptides, such as drosomycin and thanatin, are activated upon bacterial attack but also have antifungal properties (Levashina et al. 1995; Fehlbaum et al. 1996), and some

antibacterial proteins, such as lysozyme, are also active against fungi (Jaynes 1989; Jaynes et al. 1989).

1.3 Recognition of parasites and pathogens

For an immune system to defend an organism from invasion it must first recognise foreign or non-self molecules. Like vertebrates, arthropods are able to detect molecular patterns found on microbial cell surfaces (Janeway 1994) and many non-self recognition proteins have been discovered. Peptidoglycan recognition proteins are able to trigger the phenoloxidase cascade in vitro (Yoshida et al. 1996); after injection of peptidoglycan (part of the cell wall of a bacterium composed of sugars and amino acids) the fat body is stimulated to produce the same haemolymph proteins as it would in response to whole bacteria (Kanost et al. 1988a; Morishima et al. 1995), and soluble petidoglycan particles can stimulate a fat body incubated in vitro to produce antibacterial proteins (Dunn et al. 1985; Iketani and Morishima 1993). Gram-negative binding protein and β1,3-glucan and β-1,3-mannans fungal recognition proteins have been found in several invertebrate species (Ashida et al. 1986; Yoshida and Ashida 1986; Duvic and Soderhall 1992; Ochiai et al. 1992; Ochiai and Ashida 1999; Ma et al. 2000; Ma and Kanost 2000, 2001; Fabrick et al. 2003; Zhang et al. 2003). They are expressed in the immune tissues and their production is upregulated after immune challenge (Ma and Kanost 2000) and triggers the prophenoloxidase cascade discussed above in response to binding with fungal β1,3-glucan.

Lipopolysaccharides (LPS), large molecules found on the bacterial cell surface, also

stimulate the production of specific antibacterial proteins that bind with them in vivo and in vitro (Kanost et al. 1988b; Trenczek and Faye 1988; Ladendorff and Kanost 1990; Samakovlis et al. 1992; Kawasaki et al. 1993; Kobayashi et al. 1993; Lindstromdinnetz et al. 1995; Sun and Faye 1995). These have been isolated from *Periplaneta americana*, *Blaberus discoidalis* and *Galleria mellonella* and are strings of 10-30 identical protein subunits 24-30kDa in length (Jomori and Natori 1992); Kawasaki et al. 1993). Bacterial LPS is quickly removed from the haemolymph by lipid transfer proteins (Kato et al. 1994) or is taken up by granular haemocytes (Xu et al. 1995).

Drosophila have been instrumental in increasing our understanding of specificity in invertebrate immune response. The genes switched on after infection depend on whether the infection is by Gram-positive or Gram-negative bacteria (Lemaitre et al. 1997; De Gregorio et al. 2001; Irving et al. 2001) and flies that have mutations in the Toll pathway gene components are much more susceptible to Gram-positive bacteria and fungi than those without those mutations (Rutschmann et al. 2000; Rutschmann et al. 2002), whereas flies with mutations in the genes responsible for the IMD pathway are more susceptible to Gram-negative bacteria (Lemaitre et al. 1995; Leulier et al. 2003).

1.4 Immune priming

It has been argued that immune priming, where a more rapid secondary response occurs in subsequent infections, does not occur in invertebrates, firstly because they do not

have the immune system components used by vertebrates for immune priming and secondly because most invertebrates live very short lives, and so many would die of senescence before a second infection occurred (Little and Kraaijeveld 2004). Recently, however, many new receptors and signalling pathways have been discovered in invertebrate innate immune systems (Hoffmann et al. 1996; Janeway and Medzhitov 1998, 1999; Hoffmann and Reichhart 2002; Janeway and Medzhitov 2002) and there is now evidence that some immune specificity and memory may exist (Lemaitre et al. 1997; Schmid-Hempel et al. 1999; Carius et al. 2001; Kurtz and Franz 2003; Little et al. 2003; Rolff and Siva-Jothy 2003b; Vierstraete et al. 2004a; Vierstraete et al. 2004b; Witteveldt et al. 2004a; Witteveldt et al. 2004b). Priming of the invertebrate immune system after an initial exposure to a pathogen does not have to be specific (Little and Kraaijeveld 2004) and it has been shown that a generalised up regulation of the immune system occurs in response to one pathogen type (Boman et al. 1972; Rheins et al. 1980; Faulhaber and Karp 1992; Moret and Siva-Jothy 2003). However, some specificity has been shown, as an invertebrate will reject tissue transplanted from another individual while mostly tolerating transplanted tissue from its own body (Cooper 1968; Cooper and Rubilotta 1969; Hildemann et al. 1979), and not only can an invertebrate recognise and attack non-self tissue, it will react more rapidly to exposure to tissue from the same individual a few days later but not more rapidly to tissue from a different individual (Hildemann et al. 1979).

Kurtz (2005) argued that there is no reason why innate immunity should lack specificity, and that lacking the components that produce immune memory in vertebrates does not

mean they should lack memory entirely. Invertebrate immune memory could possibly occur via a combination of more general responses to a pathogen, and many immune molecules such as those in the Toll regulatory pathway, could be increased after exposure and remain at higher levels for a short time after acting as a non-specific short-term memory. However, Zhang et al. (2004) have shown that polypeptides (known as fibrinogen-related proteins) released into the haemolymph in response to some pathogens are sourced from extremely genetically diverse regions of DNA, suggesting that these proteins may form a very specific part of the invertebrate immune system.

An immune memory may give fitness benefits to those with it (Kurtz and Franz 2003; Kurtz 2005). Infections are often clumped within a population, so an individual may encounter the same pathogen more than once in its lifetime, and a rapid response to a repeat infection could be advantageous, especially if the immune memory of a pathogen common in the environment can be passed between generations (Ishikawa et al. 1992; Little et al. 2003; Little and Kraaijeveld 2004).

1.5 Density-dependent prophylaxis

It is accepted that the environment individuals find themselves in can affect their fitness. Living in a densely populated area brings an increased risk of pathogen infection and of being harmed in an aggressive encounter with another (Anderson and May 1981). It was thought that living in a crowded environment would increase disease susceptibility due to stress (Steinhaus 1958), but recent research has shown that increased investment in prophylactic disease resistance in response to the perceived increased risk of disease

exposure actually causes susceptibility to decline (Reeson et al. 1998b; Wilson and Reeson 1998; Wilson 2000; Wilson et al. 2001; Wilson et al. 2002; Wilson et al. 2003a; Cotter et al. 2004). This phenomenon is known as density-dependent prophylaxis (DDP) (Wilson & Reeson 1998). The best examples come from species that show density-dependent phase-polyphenism, such as *Schistocerca gregaria*, the desert locust, which is green at low population densities but black and yellow at high population densities (Pener and Yerushalmi 1998). The high-density phase shows increased resistance to an entomopathogenic fungus and greater haemolymph antibacterial activity than the low-density phase (Wilson et al 2002). Similar responses to density have also been shown in other insect taxa (Long 1953; Mitsui and Kunimi 1988; Goulson et al. 1995; Reeson et al. 1998a; Wilson and Reeson 1998; Barnes and Siva-Jothy 2000; Reeson et al. 2000; Cotter et al. 2004). One noteworthy observation here is that in phase-polyphenic insects the high-density phenotypes tend to be more melanic. High levels of melanin at high density have been attributed to warning colouration (Iwao 1968; Wilson 2000) and thermoregulatory benefits (Johnson et al. 1985; Goulson 1994; Gunn 1998). However, the evidence for these is ambiguous and open to more than one interpretation. Individuals with highly melanised cuticles maintain higher body temperatures than those with paler colouration (Marriott and Holloway 1998), and although a higher degree of cuticle melanisation occurs at colder temperatures (Johnson et al. 1985), this does not explain why high population densities would cause increased melanisation. Melanin is a polymer derived from amino acids, which may improve the strength of the cuticle (Wilson et al 2001) and so protect from pathogens that enter through it (Ourth and Renis 1993b; St Leger et al. 1998). Melanin is an extremely robust polymer, able to withstand

many insults (Jacobson and Emery 1991; Jacobson and Tinnell 1993; Wang and Casadevall 1994a, b; Wang et al. 1995; Doering et al. 1999). It binds to a number of proteins and inhibits the production of many lytic enzymes by microorganisms that are key to their success as a pathogen, such as proteases and chitinases (Kuo and Alexander 1967; Bull 1970). Those tobacco hornworm, *Manducta sexta*, with a melanised cuticle are able to resist penetration by a generalist entomopathogenic fungus *Metarhizium anisopliae* for 30 hours longer than those with unmelanised cuticles (St Leger et al 1988), melanistic *S. exempta* have the greatest haemolymph PO activity (Reeson et al 1998) and melanism of the cuticle is correlated with cuticle, haemolymph and midgut PO activity (Wilson et al 2001). However, no direct correlation between haemolymph PO activity and melanism was found in *T. molitor* (Barnes and Siva-Jothy 2000).

As a high level of melanin may enhance an individual's resistance to pathogens, DDP theory seems a much more likely explanation for the darker colouration of some insects at high density. The link between melanism and increased immunity has been looked at by several studies that, importantly, have controlled for density. Melanic Northern Armyworms, *Mythimna separata*, have been shown to be five times more resistant to *Nomuraea rileyi*, an entomogeneous deuteromycete fungus, than non-melanic individuals (Mitsui & Kunimi 1988), melanic Meal Worm Beetles, *Tenebrio molitor*, up to three times more resistant to the generalist entomopathogenic fungus *Metarhizium anisopliae* (Barnes and Siva-Jothy 2000) and melanic *Spodoptera littoralis* more resistant to the entomopathogenic fungus *Beauveria bassiana* (Wilson et al 2001). Melanistic *M. separata* have also been shown to be twice as resistant to

nucleopolyhedrovirus (Kunimi and Yamada 1990) with melanistic African Armyworms *Spodoptera exempta* being five times as resistant to a nucleopolyhedrosis virus (NPV) (Reeson et al 1998) and more capable of encapsulating eggs of the ectoparasitoid *Euplectrus laphygmae* than non-melanic individuals (Wilson et al 2001).

Chapter 2: General materials and methods

2.1 Study animal: *Plodia interpunctella*

Plodia interpunctella is a small (approx. 1cm length) Pyralid moth that is found globally (Tzanakakis 1959) due to commercial import and export of food items. It has been suggested that they originate in America (Heinrich 1956) with the common name Indian Meal Moth arising from their infestation of maize known as 'Indian Meal'. It was first described by Hübner in 1810 as *Tinea interpunctella*, but was redescribed by Guenee as *Plodia* in 1845 and then as *Plodia interpunctella* by Hubner in 1872 (Heinrich 1956; Cotton 1963). Its impact on economies is huge because it is a pest of stored food commodities. Processed grains, nuts, cereals, oilseeds and dried fruit are preferred, with cracked or ground produce providing a better environment than whole grains, cereals or nuts (Abdel-Rahman et al. 1968; LeCato 1976; Mbata and Osuji 1983) but pulses, cocoa, confectionary, carob and dried vegetables will also be consumed (Williams 1964; Cox and Bell 1991; Sedlacek et al. 1996; Na and Ryoo 2000; Perez-Mendoza and Aguilera-Pen 2004) and this impacts on the production and trade of stored products due to consumption and destruction of food items as well as damage to the commodity and machinery by the large amount of silk produced by these moths (Cox and Bell 1991). Eggs are laid on or near the surface of the substrate, up to 8cm deep in maize and 4cm in rye (Schmidt 1982) which usually limits the infestion to the periphery of stored products, but damage is often still extensive.

P. interpunctella are easily kept in lab conditions, perhaps due to their natural preference for stored grain, and this combined with their pest status means we know a great deal about their biology. Optimum relative humidity is 75%, with the minimum required for population growth being 20% (Cox and Bell 1991). Development is very dependent upon temperature, the ideal being around 25°C, at which development takes around 30 days. However, the temperature range for development is 18-35°C, with a development time of 60 days at 20°C and 25 days at 30°C. Although eggs will not hatch at temperatures of 15°C, once hatched larvae can survive at temperatures as low as 10°C (Cox and Bell 1991). Larvae normally pass through five instars as they develop, and although between four (McGaughey 1978) and seven (Tzanakakis 1959) have been reported it is not thought that the number of instars is temperature dependant (Hassan et al. 1962). The different instars can be distinguished by the width of the head capsule (see **table 2.1**).

Instar	**Width (mm)**	**Age of Larvae (days)**
1	0.15 – 0.20	7
2	0.28 – 0.33	10
3	0.40 – 0.45	13
4	0.60 – 0.70	16
5	0.85 – 1.15	21

Table 2.1: Head capsule width in mm and corresponding age in days after eggs laid for each instar, from Lingfield (1990).

P. interpunctella will diapause as final instar larvae as a response to overcrowding, low temperatures, continual total darkness periods, continual light periods or photoperiods of 13 hours or less. Photoperiods of over 13 hours or an increased temperature will inhibit diapause, but the specific details of when a larva will enter or leave diapause are strain-dependent (Bell and Walker 1973; Bell 1977; Bell et al. 1979; Bell 1982; Cox and Bell 1991).

Larval population density affects female mating strategy and male development. High population densities induce females to mate more frequently than those raised at low population densities, and males to develop larger abdomens and testes and release more sperm per ejaculate than when raised at low densities where they develop larger heads and eyes (Gage 1995). This suggests that males can sense population density and invest either in mate searching (large head and eyes) or in sperm competition (larger testes). The sex ratio is most commonly 1:1. However, females often emerge before males (Reyes 1969) unless there is a food shortage when males will emerge first (Podoler 1974). Adult lifespan is 7-14 days for females and 6-12 days for males if they are unfed; if fed, it increases to 10-24 days for females and 8-17 days for males (Reyes 1969). Adult females typically weigh 11-12mg and males 8-9mg upon eclosion, but this weight declines with age (Silhacek and Miller 1972).

2.2 Culture of experimental insects

Crossing 3 strains obtained from throughout the UK started the stock cultures of *P. interpunctella* used as the basis for the experiments described in this thesis, these crosses were done to increase genetic variability. 500 larvae were obtained from an outbreeding colony maintained in the laboratory at the University of Liverpool for 5 years, and before that at Imperial College at Silwood Park for 10 years, the second was sourced from the University of Sheffield and the third was a population residing at Queen Mary University of London. The resulting individuals were cultured for 14 generations before the start of any experiment.

For the inbreeding experiment *P. interpunctella* were also obtained from the Plant Science department of the Primary Industries and Fisheries (part of the Department of Employment, Economic Development & Innovation) in Queensland, Australia. These *P. interpunctella* were a wild collected population from an infestation of organic mixed nuts from one merchant. They were mixed with a population from Western Australia and maintained in the lab in Queensland for 5 years.

The stock cultures were maintained using the following protocol:

1) Newly emerged adults were collected in a large plastic wide neck PMP jar (Fischer Scientific) with the open end covered in coarse (approx. 1mm mesh) plastic netting. This was inverted over a funnel placed into a specimen tube

(Sterilin). The adult *P. interpunctella* were allowed to mate freely and lay eggs which fell through the mesh into the tube.

2) A clean jar was filled with 500mls of culture medium consisting of 10:1:1 ratio wheat bran: brewers yeast: glycerol and approximately 250 *P.interpunctella* eggs, measured by volumetric means, were added. The top of the jar was covered with fine nylon netting secured with two elastic bands.
3) These jars were maintained under a 12L:12D light regime at 27^{o}C and roughly 60% relative humidity in a controlled temperature room. One generation took 28 days under these conditions.

New stock culture jars were started on a regular basis, depending on the demand for experimental insects. After one generation old stocks were frozen for 24 hours and then discarded. Culture jars were washed and autoclaved between use and there was never any sign of disease in the colonies.

2.3 Haemolymph extraction

At the wandering stage of late 5th instar each larvae was sexed by noting for the absence or presence of testes through the cuticle (testes appear as two mid-brown spots approximately halfway down the dorsal side). They were then weighed on a Sartorius balance (BP221S Max 220g d = 0.1mg) and a 3µl sample of haemolymph extracted. This was done by piercing each individual between the final thoracic legs and the first prolegs with a fine needle and allowing a small amount of haemolymph to pool onto

Parafilm. This was then collected using a capillary tube and transferred to a 0.2ml PCR tube.

2.4 Immune function measurements

Haemocyte count: without freezing

1µl haemolymph was transferred to a 0.2ml PCR tube using a capillary tube. 7µl of phosphate buffered saline (PBS), pH 6.8, was added to each haemolymph sample and thoroughly mixed. All 8µl of this mixture was then pipetted onto a haemocytometer. All the squares on the haemocytometer were counted and summed to give an estimate of the haemocyte density for each individual.

Haemocyte count: with freezing (Cotter)

EDTA anticoagulant in phosphate buffered saline was prepared by dissolving 10mM EDTA and 10mM citric acid in 80 ml PBS, 1 M hydrochloric acid was added a drop at a time until the pH reached 7.4, and the solution was made up to 100ml with PBS. 1µl of haemolymph was transferred to a 0.2ml PCR tube using a capillary tube and thoroughly mixed with 3µl of EDTA anticoagulant in PBS. 4µl glycerol was added to each haemolymph sample to protect the cells from the effects of freezing before being placed in the freezer for storage for approximately 8 weeks. 8µl of this mixture was then pipetted onto a haemocytometer. All the squares on the centre of the haemocytometer were counted and summed to give an estimate of the haemocyte density for each individual.

Phenoloxidase Activity Assay

1µl haemolymph was transferred to a 0.2ml PCR tube using a capillary tube, 10 µl phosphate buffered saline (PBS), pH 6.8, was added and the samples frozen for 24 hours. Defrosted samples were vortexed and transferred to a 96-well U bottom microtitre plate (Sterilin) kept on ice. 100 µl of 5mM dopamine was added to each sample and the plate was placed into a spectrophotometer. The samples were incubated at 28.2^{o}C for five minutes, and then measured using a 492nm filter (Thermo Labsystems Multiskan Ascent; Ascent Software Version 2.6). They were incubated for one minute at 28.2^{o}C and then measured for a total of 20 minutes. The total change in PO activity over the twenty minute measuring period was used because preliminary experiments indicated that this provided a good estimate of the Vmax (the slope of the linear phase of the reaction).

Protein Assay

Samples were diluted 1:20 with phosphate buffered saline. Protein content of the haemolymph was measured using a general use Protein Quantification Kit (Sigma). 45µl buffer solution was added to each well of a 96-well U bottom microtitre plate (Sterilin) kept on ice. 5µl of the diluted sample was then added to the well and thoroughly mixed. After this 5µl WST-8 formazan dye solution was added to the wells and thoroughly mixed. The plate was then placed into a spectrophotometer (Thermo Labsystems Multiskan Ascent; Ascent Software Version 2.6) incubated for 30 minutes at 37 ^{o}C, and then the light absorbance was measured at 595nm.

Chapter 3: Interactions between environmental variables determine immunity

3.1 Introduction

The majority of, if not all, organisms are exposed to parasites during their lifespan and will therefore be selected to maintain an immune system for defence (Kraaijeveld et al. 2002). However, maintaining and using an immune system has been shown to be costly to the organism, leading to trade-offs between life history traits and immune defence (Sheldon and Verhulst 1996; Kraaijeveld and Godfray 1997; Webster and Woolhouse 1999; Rigby and Jokela 2000; Kraaijeveld et al. 2001; Kraaijeveld et al. 2002). Because maintaining and using an immune response is believed to be costly to the organism as a whole (Fellowes and Godfray 2000; Rolff and Siva-Jothy 2003a), it is expected that immune responses should be condition-dependent (Sheldon and Verhulst 1996). Thus, those individuals raised in good environmental conditions should have more resources to invest in their immune response than those raised in poor conditions. Evidence is accumulating to support this idea; in invertebrates, temperature and diet quality fluctuations have been shown to influence the ability of the immune system to deal with parasitic infection (Bernays and Chapman 1994; Inglis et al. 1996, 1997a, b; Blanford and Thomas 1999a, b; Moret and Schmid-Hempel 2000; Hoang 2001; Szymas and Jedruszuk 2002; Lee et al. 2006).

As many invertebrates are ectothermic, environmental temperature plays an important role in maintaining bodily functions and an unfavourable environmental temperature can inhibit the immune function of an individual (Inglis et al. 1996; Sheldon and Verhulst 1996; Inglis et al. 1997a, b; Blanford and Thomas 1999a, b) with many species of

invertebrates showing varying susceptibility to parasitoids and pathogens at different temperatures (Blumberg 1976; Kobayashi et al. 1981; Blumberg 1991; Geden 1997; Sigsgaard 2000; Frid and Myers 2002). This temperature effect has been extensively studied by researchers investigating the use of pathogens as biological control agents, and environmental temperature has been shown to affect the virulence of many pathogens in many host systems (Blumberg 1976, 1991; Carruthers et al. 1992; Hajek et al. 1993; Watson et al. 1993; Inglis et al. 1996, 1997b, a; Adamo 1998; Blanford et al. 1998; Blanford and Thomas 1999b, a; Blanford and Thomas 2000; Blanford et al. 2000; Menti et al. 2000; Sigsgaard 2000; Frid and Myers 2002; Olsen and Hoy 2002; Blanford et al. 2003; Blanford et al. 2009; Blake et al. 2010): as an example, a temperature difference as little as 2^{o}C has been shown to be the difference between survival and death of *Zonocerus variegatus* infected with the fungal pathogen *Entomoraga grylli* (Blanford et al. 2000).

As population densities increase, environmental conditions change, and there is an increased risk of infection and of aggressive encounters with others (Anderson and May 1981). To counter this, some animals are known to allocate more resources to their immune system at high densities: so-called "density-dependent prophylaxis (DPP)" (Wilson and Reeson 1998; Wilson et al. 2001; Wilson et al. 2002; Wilson et al. 2003b). The best examples of DDP come from phase-polyphenic insects; an increase in haemolymph PO activity in response to higher densities has been shown in the Lepidopteran *Spodoptera exempta* (the African army worm) (Reeson et al. 1998b; Wilson et al. 2001). Also, desert locusts (*Schistocerca gregaria*) raised under crowded

conditions are more resistant to fungal infection and have a raised haemolymph antimicrobial activity (Wilson et al. 2002).

Food quality has also been shown to influence the invertebrate immune system in a number of ways. Survival and/or immunocompetence of a host depends on the inclusion of specific nutrients in the diet which can, in some cases, be self-selected by the individual (Bernays and Chapman 1994; Mayntz and Toft 2001; Lee et al. 2003; Mayntz et al. 2005; Lee et al. 2006; Mody et al. 2007; Lee et al. 2008; Povey et al. 2009; Srygley et al. 2009). The balance of macronutrients in the diet can also mediate trade-offs between life history traits and immune function (Cotter et al. 2011). Insects that have been forced to increase their immune investment by an immunogenic challenge have reduced survival when starved (Moret and Schmid-Hempel 2000; Hoang 2001) and starved insects often have reduced immune system function (Siva-Jothy and Thompson 2002; Kubi et al. 2006; Campero et al. 2008). Food quality can also affect the number or quality of the components that make up the immune system (Szymas and Jedruszuk 2002). For example, larvae of *Rhodnius prolixus* kept on blood plasma, rather than their usual diet of whole blood, had a significantly reduced antimicrobial activity, produced significantly lower amounts of antimicrobial peptides and were less resistant to bacterial infection (Feder et al. 1997). Bacterially-challenged *Spodoptera exempta* larvae have greatest survival on a diet rich in protein with survival decreasing as protein levels of the diet decrease (Povey et al. 2009) and PO activity in the Mormon cricket *Anabrus simplex* is reduced by a diet limited in protein (Srygley et al. 2009).

In addition to effects from limited availability of protein, the availability of micronutrients can also influence immune investment; for example, an important function of diet and feeding is the intake of antioxidants. Melanisation reactions and the process of encapsulating an injury site or an intruder produce free radicals that can be responsible for invertebrate cell damage (Nappi et al. 1995b; von Schantz et al. 1999; Sadd and Siva-Jothy 2006) and intake of antioxidants through diet can ameliorate this cost of mounting an immune response (Johnson and Felton 2001; Ojala et al. 2005; Babin et al. 2010).

Whether environmental factors interact to produce synergistic effects on immune response is an important question because it will be rare for one environmental factor to vary completely independently of others, meaning that predictions from laboratory studies where animals are kept in otherwise constant environments with only one factor varying might produce unreliable predictions of immune investment in the field. To address this issue I compared two immune system indicators (haemocyte count and phenoloxidase activity) among groups of animals raised in different environmental conditions. The temperature, density and food quality in which the *P. interpunctella* were raised was manipulated to try to determine the effects of interactions between these environmental variables on immunity.

3.2 Methods

3.2.1 Experimental design

The variables manipulated were temperature (30^{o}C versus 27^{o}C), increasing the amount of wheat bran in the diet (normal diet versus poor diet with half as much brewers yeast (a protein source in the diet) and glycerol (a sugar source)) and density (3 individuals per group versus 10 individuals per group). These were varied in a fully factorial design for a total of eight treatment combinations. 50 groups of larvae were reared per treatment.

For the treatments with a raised temperature the Petri dishes were placed in a single layer on a thermostatically controlled heat mat (BioGreen HMT 060-200/GB 263W) covered with a layer of aluminum foil to help distribute heat evenly across the mat. Unheated treatments were placed on a similar mat that was not turned on, the temperature in the CT room was measured and kept constant. This design obviously introduces a degree of pseudoreplication but we tried to reduce any confounding effects by moving the Petri dishes around on the mats several times during rearing and by keeping the heated and unheated mats next to each other on a single lab bench.

Haemolymph samples were collected from 379 *P. interpunctella*, 120 and 113 from the high density treatments, high quality and low quality food respectively, and 72 and 70 from the low density treatments, high quality and low quality food respectively. All

larvae were weighed and a 2-3µl sample of haemolymph extracted for haemocyte counts and phenolxidase assays.

3.2.2 Analysis of data

In a design like this the unit of replication is the group, rather than the individual larva so mean values were calculated for each Petri dish and the analysis carried out on these. General linear models were fitted to the immune function data with temperature, density and food quality plus all interactions as explanatory variables, plus weight as a covariate. PO activity was log +1 transformed to reduce heteroscedasticity. Non-significant terms were removed following deletion tests (Crawley 2002) to leave a minimal adequate model. All analyses were performed using R version 2.8.1 for Macintosh.

3.3 Results

For haemocyte count there was a significant 3-way interaction between density, food quality and temperature ($F_{1,83} = 7.73$, $P = 0.007$; Fig. 3.1 a, b) but weight was not significant and was removed ($F_{1,83} = 0.30$). Three significant 2-way interactions: food quality and density ($F_{1,56} = 15.59$, $P < 0.001$; Fig. 3.2a), density and temperature ($F_{1,56} = 20.13$, $P < 0.001$; Fig. 3.2b) and food quality and temperature ($F_{1,56} = 12.06$, $P = 0.001$; Fig. 3.2c) affect PO activity with weight once again being non-significant and removed ($F_{1,56} = 0.12$).

When weight was analysed separately as a response variable the minimal model retained two significant 2-way interactions; temperature and density ($F_{1,86} = 8.01$, $P = 0.005$; Fig. 3.3a) and temperature and food quality ($F_{1,86} = 17.07$, $P < 0.001$; Fig. 3.3a).

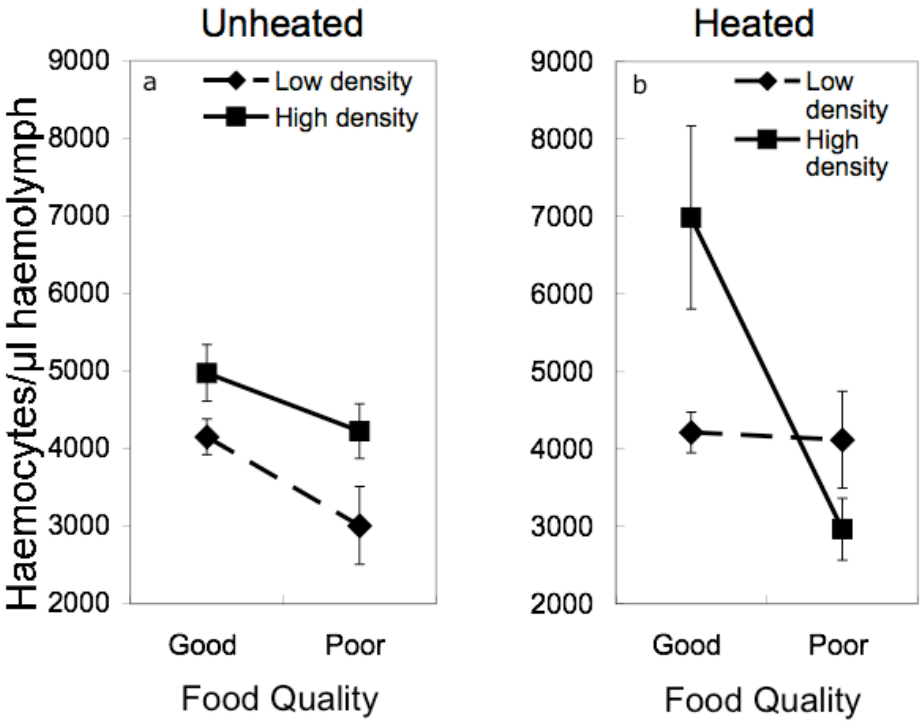

Figure 3.1: Mean haemocyte density showing the significant 3-way interaction between density, food quality and haemocyte number. (a) The unheated treatment of 27°C, showing the effects of density (high density 10 individuals per Petri dish, low density 3 individuals per Petri dish) and food quality (high food quality contained twice the amount of brewers yeast and glycerol per 100g wheat bran than the poor quality food); and (b) the effects of density and food quality in the heated treatment (30°C). Error bars are one standard error.

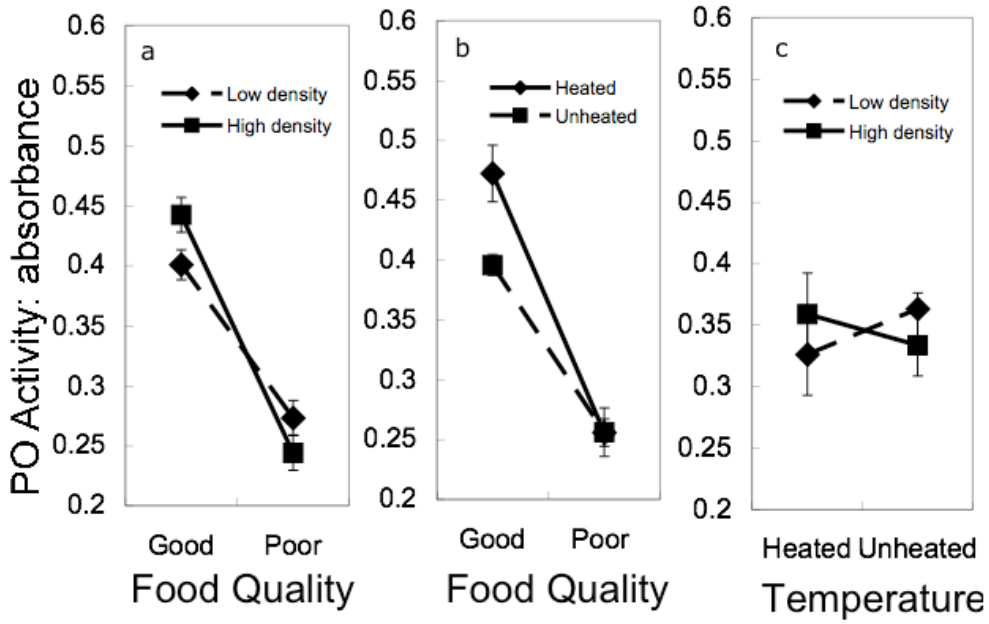

Figure 3.2: Mean phenoloxidase (PO) activity showing the three significant 2-way interactions between (a) food quality and density, (b) food quality and temperature and (c) density and temperature. Error bars are one standard error.

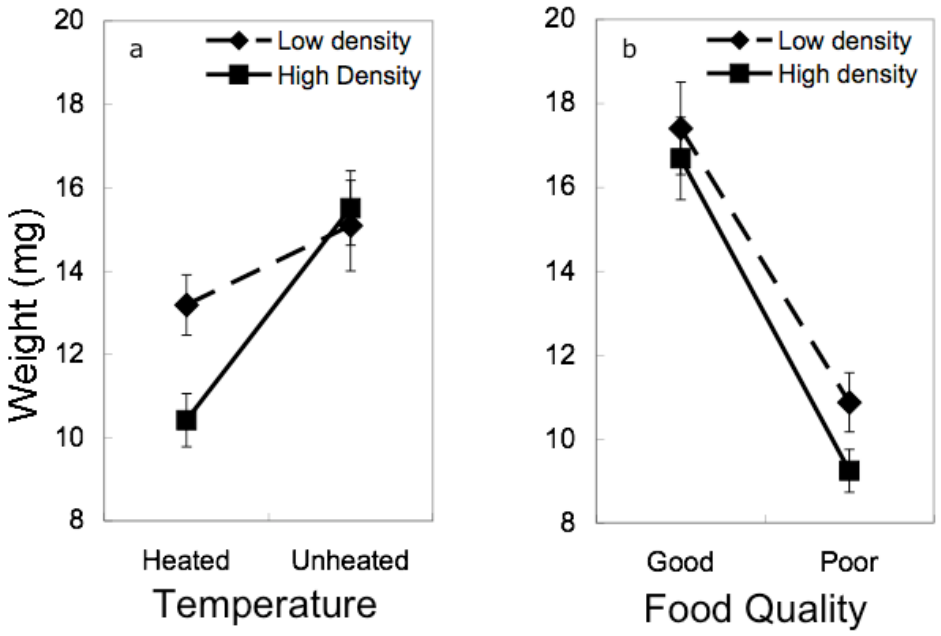

Figure 3.3: Mean weight showing the two significant 2-way interactions between (a) temperature and density and (b) density and food quality. Error bars are one standard error.

3.4 Discussion

The aim of this study was to gain a better understanding of how different environmental factors interact to affect immune system function in a model insect species, *P. interpunctella*. The results are consistent with previous work that has shown that environmental factors have a profound effect on immune system parameters and extend our understanding by demonstrating that the effects of these factors are themselves critically dependent on other environmental variables.

3.4.1 Temperature

In some cases, increased temperature led to higher scores for immune system indicators. When density was high or food quality was good higher temperatures led to higher PO activity (Figs 2b and 2c), but higher temperatures were only associated with higher haemocyte counts when the food quality was good and density high (Figs 1a and 1b). When the density was high and the food quality good the low temperature treatment had a substantially higher haemocyte count than the high temperature treatment. Some invertebrates can behaviourally induce 'fever' when they are infected (Karban 1988; McClain et al. 1988; Watson et al. 1993; Adamo 1998; Blanford et al. 1998) but the data suggest that the fever either does not increase haemocyte count or did not in this case or at these temperatures. A gradual increase in temperature from 10 to 20^{o}C caused a significant increase in haemocyte number in the crab *Carcinus maenus* (Truscott and White 1990) and total haemocyte number was found to be significantly higher in the two crayfish species *Pacifastacus leniusculus* and *Astacus astacus* when kept at 18 ^{o}C

rather than 4 °C (Jiravanichpaisal et al. 2004). Our data suggest that increased temperature only leads to higher immune reactivity under certain circumstances, and that different components of the immune system respond differently to temperature.

Even when increased temperature does lead to an improved immune response there can still be a cost to the animal: insects with an increased body temperature are known to suffer reduced survival and fecundity (Boorstein and Ewald 1987). In our study the *P. interpunctella* larvae reached a much smaller body size at the higher temperature, perhaps due to faster development rates (Johnson et al. 1992). Since fecundity increases with body size across many insect taxa (Honek 1993) it is possible that, although in some cases these animals had increased immune function at higher temperatures, their smaller body size would result in a cost for the individual in the form of reduced fecundity. However, it should be noted that Johnson, Wofford & Whitehand (1992) showed that *P. interpunctella* raised on a bran diet at 31.7 °C produced more progeny than those raised at 25 °C and 28.3 °C.

Our high-temperature treatment was 3 °C warmer than the unheated treatment, an increase that falls within current predictions for anthropogenic climate change on Earth (Metz et al. 2007). Recently, studies have looked at how this predicted change in environmental temperature might affect host/pathogen dynamics and infection rates, with some showing an increase in pathogen virulence due to accelerated growth and increased transmission rates (Harvell et al. 2002; Mydlarz et al. 2006), while others have shown a reduction in virulence, due to the parasite no longer being at optimum

temperature or to improved host immune system function(Blanford et al. 2003). Garner, Rowcliffe & Fisher (2010) have shown that common toads (*Bufo bufo*) who spend an overwintering period in a warmer temperature regime (36 days at 8^{o}C, 11 days at 4^{o}C and then another 36 days at 8^{o}C) have a greater chance of infection than those raised in a cooler temperature regime that mimicked their natural environment (16 days at 8^{o}C, 51 days at 4^{o}C and then another 16 days at 8^{o}C). Our study shows that a small temperature increase produces strong effects on invertebrate immune system function, which suggests that the predicted rise in global temperature, no matter how small, has the potential to change host/pathogen dynamics across natural environments.

3.4.2 Food quality

In general, food quality had a substantial effect on larval immune system function. Larvae raised on the good quality diet had higher PO activity in every case and higher haemocyte counts in every case except in the unheated:low density treatment. This is consistent with resource allocation theories, where the resources needed for one fitness component must be traded-off against resources needed for another (Sheldon and Verhulst 1996). Our data suggest that a good quality diet may have provided adequate resources to maintain immune activity, while the poor quality diet resulted in immune activity being compromised. As with temperature and density, however, the effect of diet quality on immune reactivity is not a simple additive increase and there are strong interactions with other environmental variables, the effect of density in the lower temperature treatments being particularly pronounced. As was the case with temperature, the two components of the immune system measured here responded

differently to food quality, although the differences are not as marked as they were for temperature, with the differences mainly being in the magnitude, not the direction of the response.

3.4.3 Density

The density-dependent prophylaxis (DDP) hypothesis predicts that there should be an increase in investment in immunity at high population densities, a phenomenon that has been described from a number of other insect systems (Wilson and Reeson 1998; Wilson 2000; Wilson et al. 2002; Wilson et al. 2003b). In this case it was found in some treatment combinations only. When the food quality was good, high densities were associated with higher haemocyte counts and with higher PO activity (Figs 1a, 1b, 2a). When food quality was poor, however, the situation was reversed, with the low-density treatments expressing higher PO activity (Fig. 2a) and, in the case of the normal temperature treatment, higher haemocyte counts as well (Fig. 1b). It appears that when resources are plentiful the larvae invest more into immunity at higher densities, but that the combined impact of both high density and poor food has the opposite effect due, perhaps, to any potential for immune increase being limited by the lack of resources or the result of other trade-offs due to density.

Density effects also depend on temperature in a complex way. In the case of PO activity high density led to higher PO activity in the heated treatment than in the unheated treatment, and the reverse at low densities, with the unheated:low density treatments having higher PO activity than the heated:low density treatments. In the case of

haemocyte count the interaction between food quality and density was only noticeable in the unheated treatments, and when the temperature was raised high-density animals had higher haemocyte counts than low-density ones even when the food quality was poor.

3.4.4 Weight

Good food quality and low density led to a much higher larval weight being achieved than poor food quality and high density. An additional increase in weight occurred in the unheated treatment, with those raised at low and high densities having similar weights. In the heated treatment however, those larvae raised at low density reached much grater weights than those raised at high densities in the heated treatment. As food was provided *ad libitum* there should not have been any direct competition for food, so this effect of density is likely to be caused by indirect competition; for example, those raised at high density may have spent more time and energy on aggressive encounters with others rather than on feeding and growing.

This study demonstrates the importance of considering interactions between different environmental variables when trying to understand the evolutionary ecology of immune responses. When an individual variable is studied in isolation important interactions and their effects may be overlooked. Such interactions could cause laboratory studies, where animals are kept in otherwise constant environments, to produce unreliable predictions of immune function in natural systems. Further research into how multiple environmental variables may interact to produce an entirely different effect from those

variables in isolation may help to increase our understanding of how important interactions are to immune responses.

Chapter 4: Parental effects on offspring immunity

4.1 Introduction

Environmental factors can have profound effects on individual fitness. As has been shown in chapter 2, unfavourable environmental temperatures can inhibit the immune function of an individual (Inglis et al. 1996, 1997a, b; Blanford et al. 1998; Blanford and Thomas 1999a, b; Blanford et al. 2000; Blanford et al. 2003), high population densities bring increased risk of infection and of aggressive encounters with others (Anderson and May 1981; Wilson and Reeson 1998; Wilson et al. 2001; Wilson et al. 2003b) and food quality can affect growth, reproduction, body condition (Boersma and Vijverberg 1995, 1996) and the number or quality of the components that make up the immune system (Feder et al. 1997; Szymas and Jedruszuk 2002; Ojala et al. 2005). Individuals raised on a low quality diet have increased mortality rates, lower average lifetime reproduction (Carey et al. 2002) and are often smaller and older at metamorphosis and maturity (Alford and Harris 1988; Stamp and Bowers 1990; Boggs and Ross 1993). Females raised on a poor quality diet may sometimes produce a smaller number of larger eggs and/or fewer, but better provisioned, offspring (Strathmann 1985; Sinervo and DeNardo 1996). Alternatively, the nutritional content of the eggs may be lowered resulting in smaller offspring, both at larval and postlarval stages (Sinervo and Mcedward 1988; Emlet and HoeghGuldberg 1997; Bertram and Strathmann 1998). It is expected that an individual raised in good environmental conditions should achieve a better body condition and, as a result, have more resources available to invest in its immune

response and in its reproductive success than an individual raised in poor conditions (Westneat and Birkhead 1998).

An unfavourable environment does not only directly affect the fitness of those animals that experience it, but can also indirectly affect the fitness of their offspring via parental effects (Mousseau and Fox 1998; Pal and Miklos 1999). Environmental parental effects are those features derived from the environment experienced by the parents and/or their condition at the time of reproduction and not from nuclear genes (Bernardo 1996; Rossiter 1996) and it has been suggested that these effects could alter population dynamics and evolution (Willham 1972; Hanrahan 1976; Rossiter 1991).

When non-genetic information is passed further than one generation it is known as a 'permanent' (Willham 1972) or 'persistent' (Riska et al. 1985) environmental parental effect, or 'environmental inheritance' (Riska et al. 1985). Kirkpatrick and Lande (1989) clarified this further by using the term 'maternal inheritance' for the transmission of non-genetic information from mother to offspring and 'maternal selection' for the effect of this on the fitness of her offspring, and Lombardi (1996) used the term 'maternal influence' to refer to the source of the effect and not the impact on the offspring phenotype that it has.

Maternal and paternal effects include those derived from the environment endured by the parents and/or their condition at the time of reproduction (Bernardo 1996) as well as nuclear and cytoplasmic genes passed from the parents to the offspring (Kondo et al.

1990; Wade and Beeman 1994). If the environment that a parental generation experiences is a predictor of the environment their offspring will find themselves in, there will be selection on the parental generations to optimise offspring phenotype for success in that environment (Mousseau and Dingle 1991; Mousseau and Fox 1998; Fox and Czesak 2000; Fox and Savalli 2000; Gilchrist and Huey 2001; Holbrook and Schal 2004). For example, *Daphnia pulex* offspring whose mothers were exposed to chemicals released by a predator are born with defensive structures that protect them against that predator (Tollrian 1995). If the parental generation are in unfavourable conditions this may restrict their ability to invest optimally in their offspring, or if an individual has to devote a larger proportion of its available resources to somatic maintenance, it may change the optimal investment per offspring. If the environment is unstable there may be selection for an increase in offspring variation to provide a more optimal resource distribution (Brockelman 1975) but the ability to control the investment in offspring may be affected by factors such as pleiotropy and the very environmental effects they are trying to overcome during offspring development (Mcginley et al. 1987).

Regarding parental effects on immunity, transgenerational immune priming is generally accepted to occur in vertebrates through the passage of antibodies between mother and offspring (Grindstaff et al. 2002; Grindstaff et al. 2003), but until recently there was little, and controversial, evidence for it occurring in invertebrates (Little et al. 2003). Sadd et al. (2005) used the bumblebee, *Bombus terrestris*, to demonstrate that offspring whose mother had been challenged with heat-killed bacteria in saline had significantly

higher antibacterial activity than those whose mother had received injections of saline only. This increase in offspring antibacterial activity is a very specific response to the immune challenge experienced by the mother, and Roth et al. (2009) discovered that not only mothers have the ability to prime their offspring for any immune challenges they are likely to face, but fathers can do this as well.

Effects of diet quality on paternal provisioning for offspring have been reported in many systems (Rossiter 1996; Mousseau and Fox 1998; Vahed 1998). Egg size is often correlated with nutritional content of the egg (Bondari et al. 1978; Bolton 1991), which can affect the fitness of the offspring during development (Roosenburg and Kelley 1996).

Where a species does not perform paternal care of the offspring it might be assumed that paternal effects are less important or absent, but there is mounting evidence that this is not always the case (Bonduriansky and Head 2007; Hunt and Simmons 2007). In *Drosophila*, for example, the environmental temperature that a male experiences affects wing length (Huey et al. 1995), cold resistance (Watson and Hoffmann 1996), productivity and fecundity (Magiafoglou and Hoffmann 2003) in his offspring, and in the fly *Telostylinus angusticollis* males in good condition produce larger offspring, with their sons having increased mating success and their daughters increased fecundity than those from low condition males (Bonduriansky & Head 2007). Such effects may be mediated by female provisioning of the eggs in response to the quality of the male, and Burley (1988) put forward the idea of differential allocation (DA), where a female

should increase her investment in the offspring if she has mated with a high quality male, as mate quality reflects the returns on her investment in terms of future reproductive success. However, this prediction was contested (Bluhm and Gowaty 2004; Gowaty 2008) and the reproductive compensation (RC) hypothesis proposed. This predicts that females should increases their investment in offspring when paired to a low quality male to attempt to negate any negative effects his low quality may have on their offspring. It is reasonable to expect that DA and RC will occur in different systems; DA was developed using a bird species as a model system that exhibits both maternal and paternal parental care (Burley 1988), but it has been shown in many vertebrate and invertebrate species that also demonstrate traditional paternal care and in those that do not (Petrie and Williams 1993; Wedell 1996; Cunningham and Russell 2000; Kolm 2001; Head et al. 2006), while RC has been shown in cases were a female has little or no mate choice (Bluhm & Gowaty 2004; Gowaty 2007; Gowaty *et al* 2007; Gowaty 2008). Conversely, some studies have shown no effect of male quality on maternal provisioning at all (Oksanen et al. 1999; Mazuc et al. 2003; Rutstein et al. 2004; Reaney and Knell 2010). Harris and Uller (2009) modelled female reproductive investment using a state-based approach based on the resources a female has at a particular time to invest in her reproduction and in her own maintenance. They found that DA (increasing investment when mated to a high quality mate) was the optimal strategy for females in their model; RC was only predicted when there was a low impact of parental investment on offspring quality. They also discovered a relationship between female state (her condition, body size, age etc) and the level of investment in her offspring in relation to male quality. The effects of diet quality on the immune

system have been extensively studied (reviewed in chapters 1 and 2), and while immune priming and parental environmental effects have been the focus of some research, immune changes arising from inherited environmental effects are less well known, especially paternal effects. To address the question of how these effects on the immune system caused by diet quality are passed down from parents to offspring, the Indian Meal Moth, *Plodia interpunctella*, (Hübner) was used as a model system. We compared two immune system indicators (haemocyte count and phenoloxidase activity) between groups of animals raised in different environmental conditions, and their offspring. The density of haemocytes reflects the ability of an individual to encapsulate a wound site or intruder, as well as resist bacterial and viral attack (Eslin & Prévost 1996), while phenoloxidase (PO) is the enzyme responsible for producing the pigment melanin. Melanisation is a key part of encapsulation and PO activity has been shown to correlate with the ability of many insects to resist attack by pathogens (Ourth and Renis 1993a; Washburn et al. 1996; Barnes and Siva-Jothy 2000; Wilson et al. 2001; Cotter et al. 2004).

The environmental variable manipulated was food quality, the normal diet being 10:1:1 ratio wheat bran: brewers yeast: glycerol whereas a poor diet contained half as much yeast and glycerol to wheat bran. Adults were reared on either normal or poor food, mated and then their offspring were divided into two groups that were also reared on normal or poor food, giving a total of eight treatment combinations. 50 groups of larvae were reared per treatment.

4.2 Experimental design

4.2.1 The parental generation

Over 300 adult moths from the stock culture were placed in one jar and all eggs collected. Each egg was then allocated to one of two food treatments, normal or poor food. Eggs were placed individually into small 100ml plastic pots and provided with *ad libitum* food of the appropriate quality. Larvae were raised in a 27^{o}C controlled temperature room under a 12:12 light:dark cycle. These were the parental generation. At 5th instar each larva~~e~~ was sexed (on the basis of the absence or presence of testes, which are visible through the cuticle) weighed on a Sartorius balance and a 3µl sample of haemolymph was extracted.

The larvae were then transferred back into their original plastic pot. Within 12 hours of emerging as adults, individuals were chilled and photographed using a dissection microscope.

4.2.2 Mating pairs

Following eclosion, experimental females were randomly allocated to an experimental male raised on either normal or low quality food. The pair were placed in a clean 55mm Petri dish in a temperature-controlled room at 27^{o}C and left to mate and lay eggs for 48 hours, after which the adults were removed from the dish and placed in a new clean

Petri dish in the temperature-controlled room and allowed to mate and lay eggs until the female died.

4.2.3 Offspring

The eggs laid per pair in the first two days were counted and 20 eggs from each pair were allocated to the poor food quality treatment and 20 to the normal food quality treatment. Each was placed individually into a plastic pot with an *ad lib.* diet of the appropriate food quality. These were kept in the controlled temperature room until they had reached late 5^{th} instar when each larva was weighed and had a sample of haemolymph extracted as before.

Haemolymph samples and life history measurements were taken from 317 parental generation *P. interpunctella* at late 5^{th} instar, 69 males and 84 females from the high quality food treatment and 89 males and 75 females from the low quality food treatment, and further samples were taken from 2722 offspring generation *P. interpunctella*, 1372 from the high quality food treatment and 1349 from the low quality food treatment.

4.3 Analysis of results

4.3.1 Statistical analysis of haemocyte count

The haemocyte count data were square-root transformed to give an acceptable error distribution prior to mixed model analysis with family as a random factor. Block was

included as a fixed effect due to the rather low number of factor levels (7). Maternal treatment, paternal treatment, offspring sex and offspring treatment were included in the initial model as explanatory factors plus their two-way interactions, and offspring weight, maternal haemocyte count and paternal haemocyte count were also included. Models were initially fitted using maximum likelihood to allow LR tests to compare model fit (Crawley 2002; Zuur 2009) and a minimal adequate model produced which was then re-fitted using REML.

4.3.2 Statistical analysis of PO data

The PO data were analysed as for haemocyte count, except that paternal and maternal PO activity were substituted for paternal and maternal haemocyte count in the initial model. After initial model fitting and data exploration the interaction terms between offspring treatment and maternal and paternal PO activity were added to the model.

4.3.3 Statistical analysis of survival data

An initial generalised linear mixed model with binomial errors was fitted by the Laplace approximation with maternal treatment, paternal treatment and offspring treatment as fixed effects plus their two-way interactions, plus maternal and paternal weight as continuous fixed effects. Family was fitted as a random factor and block as a fixed effect because of the low number of factor levels.

On the basis of both the Wald tests and LR tests block, paternal size and the maternal treatment by offspring treatment interaction were strongly supported for retention. Both

tests support the removal of maternal size and the paternal treatment by offspring treatment interaction so a candidate model with both of these terms removed was tested. All remaining terms were supported by significant z-scores, so that was the final model.

4.3.4 Statistical analysis of egg length data

An initial general linear mixed model was fitted with maternal treatment, paternal treatment as fixed effects plus their two-way interactions, plus maternal and paternal weight as continuous fixed effects. Family was fitted as a random factor and block as a fixed effect because of the low number of factor levels.

All analyses were performed using R version 2.8.1 for Macintosh.

4.4 Results

4.4.1 Haemocyte count

The minimal model for haemocyte count retained two interactions, one between paternal and maternal diet quality ($LR_{1,131} = 7.77$, $P = 0.0053$; Fig 4.1) and one between maternal diet quality and offspring diet quality ($LR_{1,2517} = 9.50$, $P = 0.0021$; Fig 4.1). There were also significant main effects of block ($LR_{1,2517} = 21.73$, $P = 0.0014$) and offspring sex ($LR_{1,2517} = 30.60$, $P < 0.0001$; Fig 4.2). Haemocyte count is highest in the offspring whose parents were both fed a good quality diet and lowest in those whose parents were both fed a poor quality diet. Just one parent fed on a poor quality diet is enough to lower the haemocyte count to a similar amount regardless of which parent. Offspring fed on a good quality diet have a higher number of haemocytes than those fed a poor

quality diet. However, maternal diet interacts with offspring diet to raise haemocyte count further only when both the mother and offspring were fed a good quality diet.

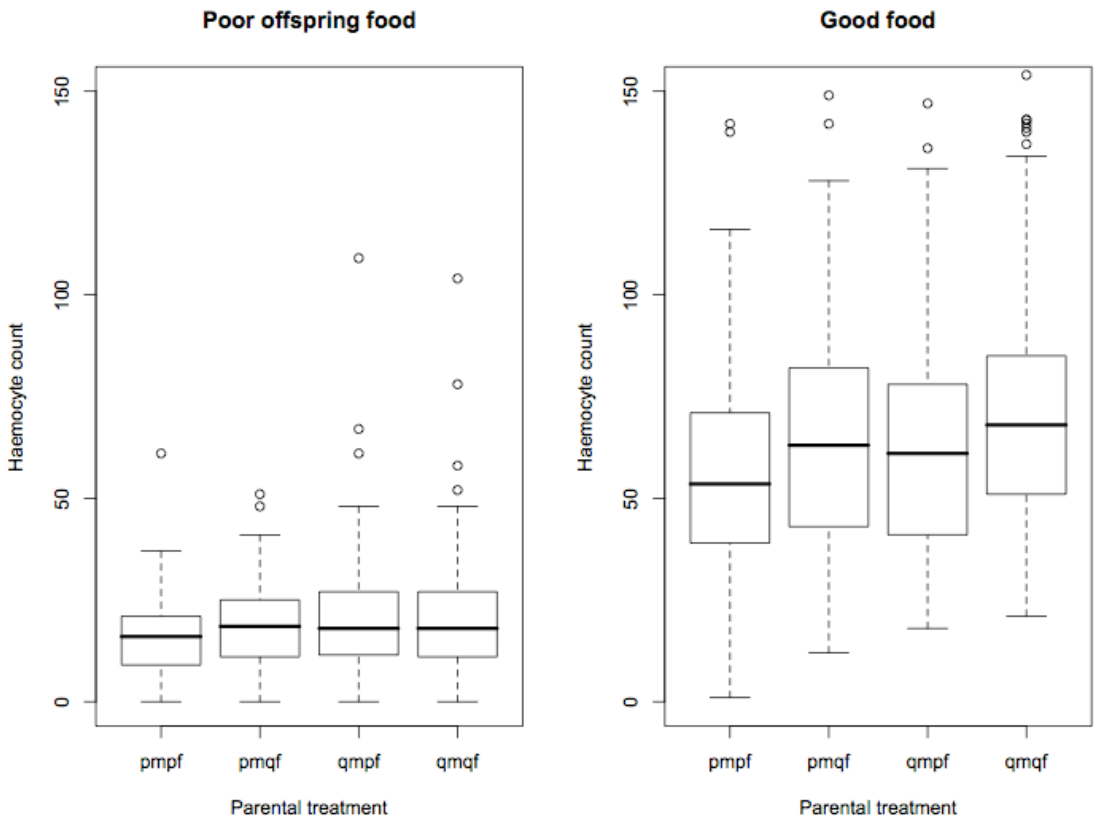

Figure 4.1: Haemocyte count for each offspring food treatment showing the interactions between maternal diet quality and paternal diet quality and between maternal diet quality and offspring diet quality. The bold lines show the median, the boxes the first quartile and the circles indicate outlying data points.

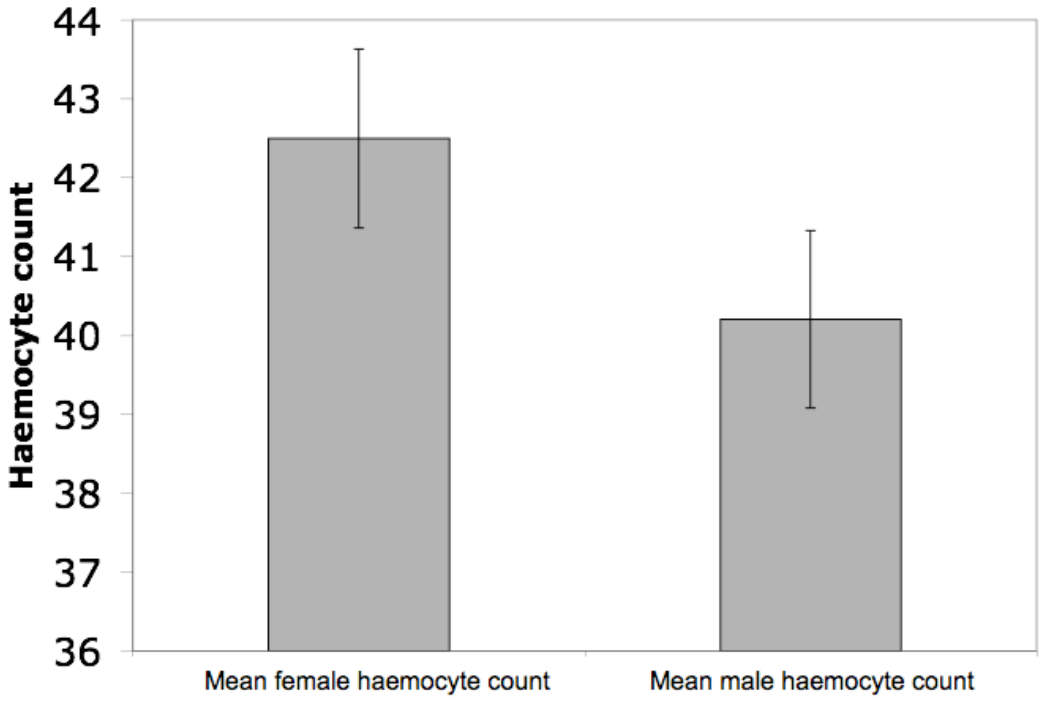

Figure 4.2: Haemocyte count for each gender showing the difference in haemocyte counts in offspring generation. Error bars are one standard error.

4.4.2 PO activity

The minimal model for PO activity retained interactions between paternal and offspring diet quality ($LR = 15.8$, $P = 0.0001$; Fig 4.3), maternal and offspring diet quality ($LR = 38.4$, $P = <0.0001$; Fig 4.3), paternal PO activity and offspring diet quality ($LR = 93.6$, $P = <0.0001$; Fig 4.4a) and maternal PO activity and offspring diet quality ($LR = 148.5$, $P = <0.0001$; Fig 4.4b). Offspring weight also slightly affected PO activity ($LR = 4.15$, $P = 0.0416$; Fig 4.5), the heavier the larva the higher their PO activity. PO activity is highest in the offspring whose parents were both fed a good quality diet (mean Vmax of phenoloxidase oxidation of dopamine of 0.452, SE 0.00191) and lowest in those whose parents were both fed a poor quality diet (mean Vmax of phenoloxidase oxidation of dopamine of absorbance of 0.32, SE 0.00216). Just one parent fed on a poor quality diet is enough to lower offspring PO activity, with maternal diet quality having more of an effect than paternal diet quality. Higher parental PO activity leads to higher offspring PO activity, with maternal PO activity having more of an influence than paternal PO activity. However, parental diet interacts with offspring diet to raise it further when the offspring are given a good quality diet.

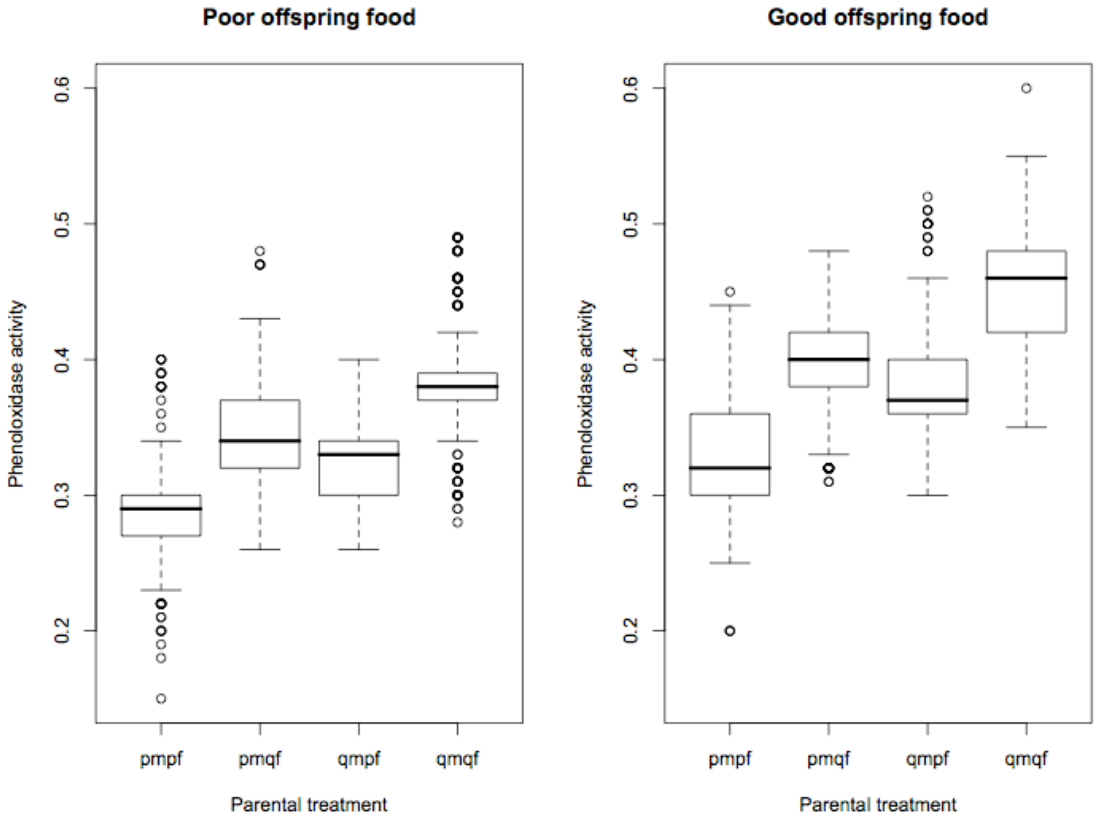

Figure 4.3: Phenoloxidase activity for each offspring food treatment showing the interactions between maternal diet quality and paternal diet quality and between paternal diet quality and offspring diet quality. The bold lines show the median, the boxes the first quartile and the circles indicate outlying data points.

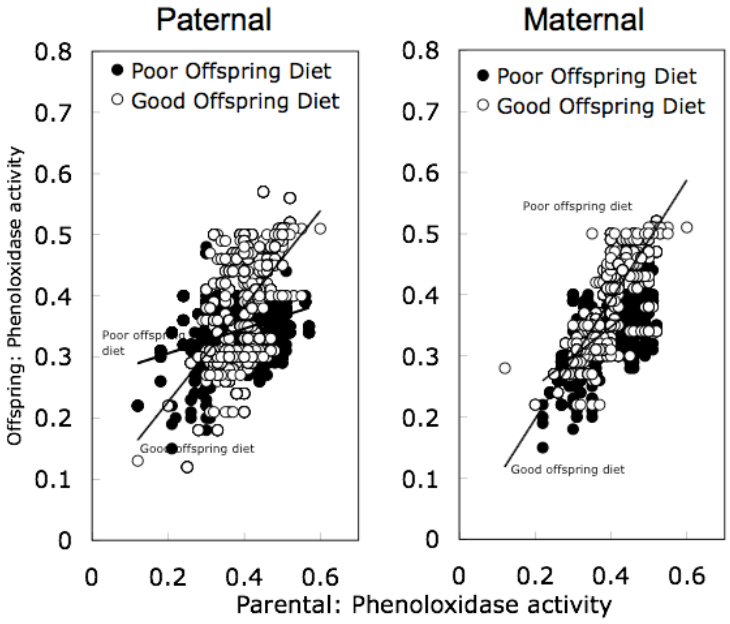

Figure 4.4: phenoloxidase activity for the interaction between paternal PO activity and offspring diet quality and maternal PO activity and offspring diet quality.

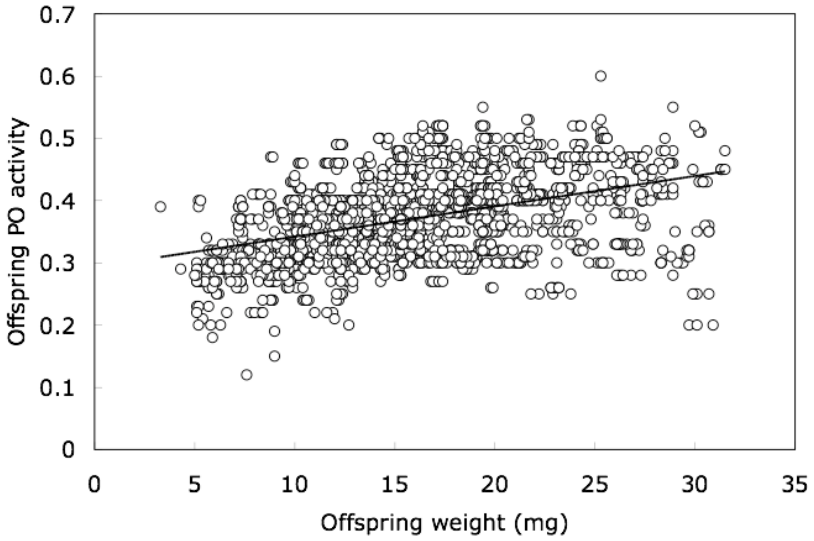

Figure 4.5: The effect of offspring weight on PO activity.

4.4.3 Offspring survival

The minimal model for offspring survival retained interactions between paternal and maternal diet quality (Z= 5.581, Pr = <0.0001; Fig 4.6) and between maternal and offspring diet quality (Z= 2.301, Pr = 0.0214; Fig 4.7). There was also an effect of paternal size (Z= 2.301, Pr = 0.0214; Fig 4.8).

More offspring fed a good quality diet survived to 5th instar than those fed a poor quality diet. Those whose mothers were fed a good quality diet had a greater chance of survival than those whose mothers were fed a poor quality diet; this was also true for paternal diet with those that had a larger father also having a greater chance of survival.

Survival was highest in the offspring whose parents were both fed a good quality diet and lowest in those whose parents were both fed a poor quality diet. Just one parent fed on a poor quality diet is enough to lower the survival, with poor paternal diet quality having more of an effect on survival than maternal diet quality. There was also an interaction between maternal diet quality and offspring diet quality, survival being greatly increased if the mother had a good quality diet and still further increased if the offspring had a good quality diet as well.

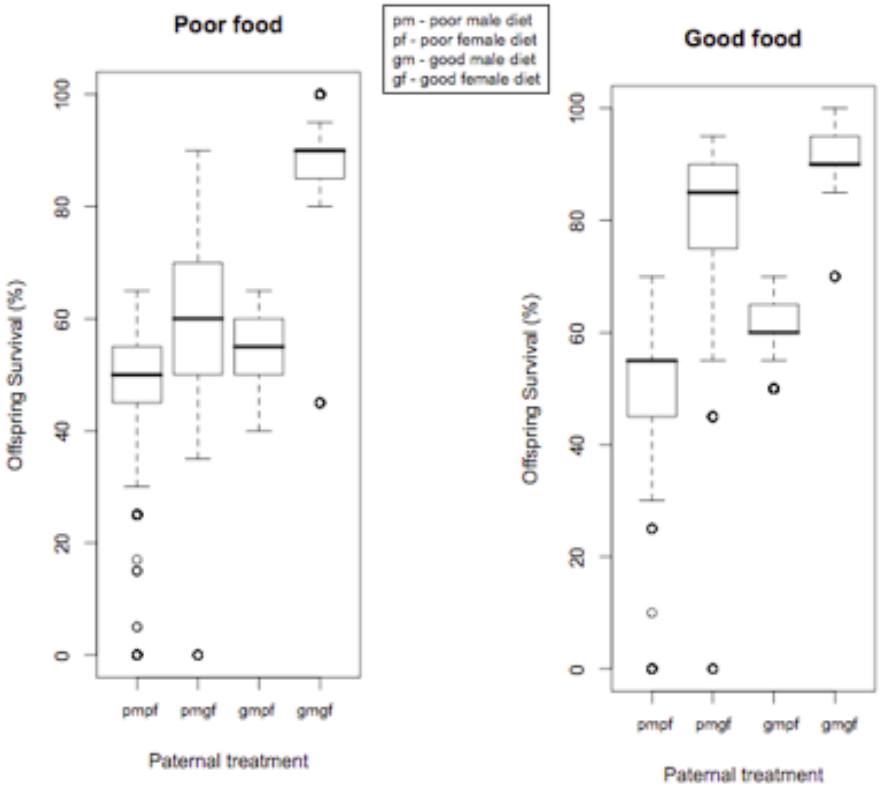

Figure 4.6: The interaction between paternal treatment and offspring diet quality and its effect on offspring survival. The bold lines show the median, the boxes the first quartile and the circles indicate outlying data points.

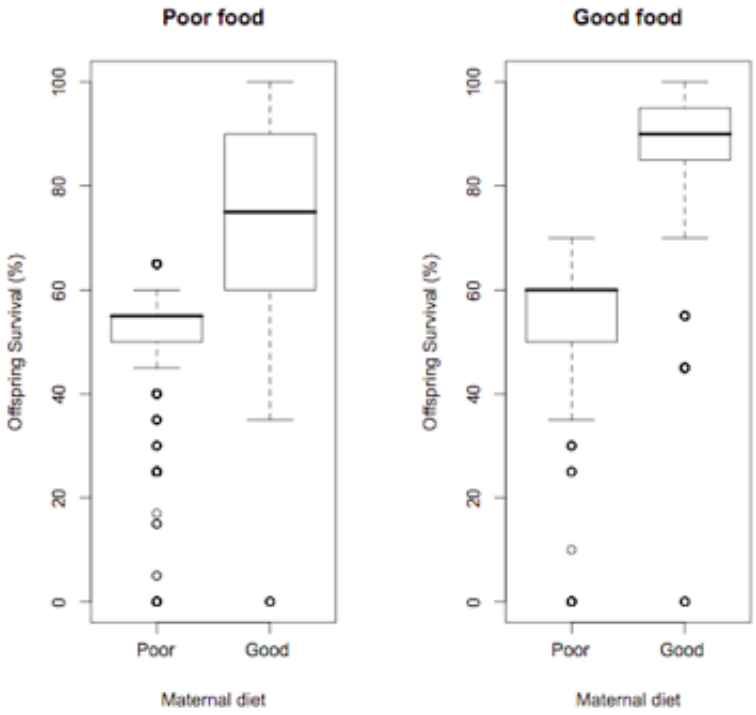

Figure 4.7: The effect of maternal diet quality and offspring diet quality on offspring survival

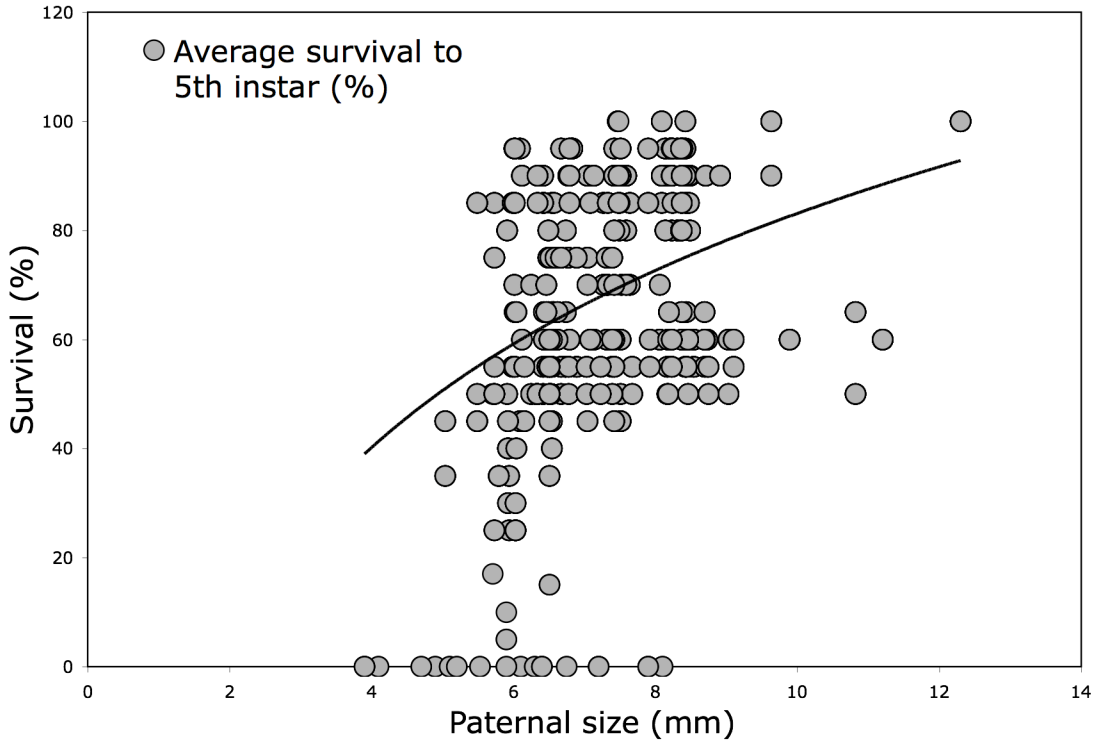

Figure 4.8: The influence of paternal size on offspring survival.

4.4.4 Egg size

A highly significant interaction was found between paternal treatment and maternal size ($F_{1,136} = 19.892$, $P < 0.001$; Fig. 4.9). There was no effect of female size on egg length when the females were mated to a male that had received a good quality diet. The eggs from females mated to a male that had received a poor quality diet showed a strong effect of maternal size; larger females laid larger eggs and smaller females laid smaller eggs.

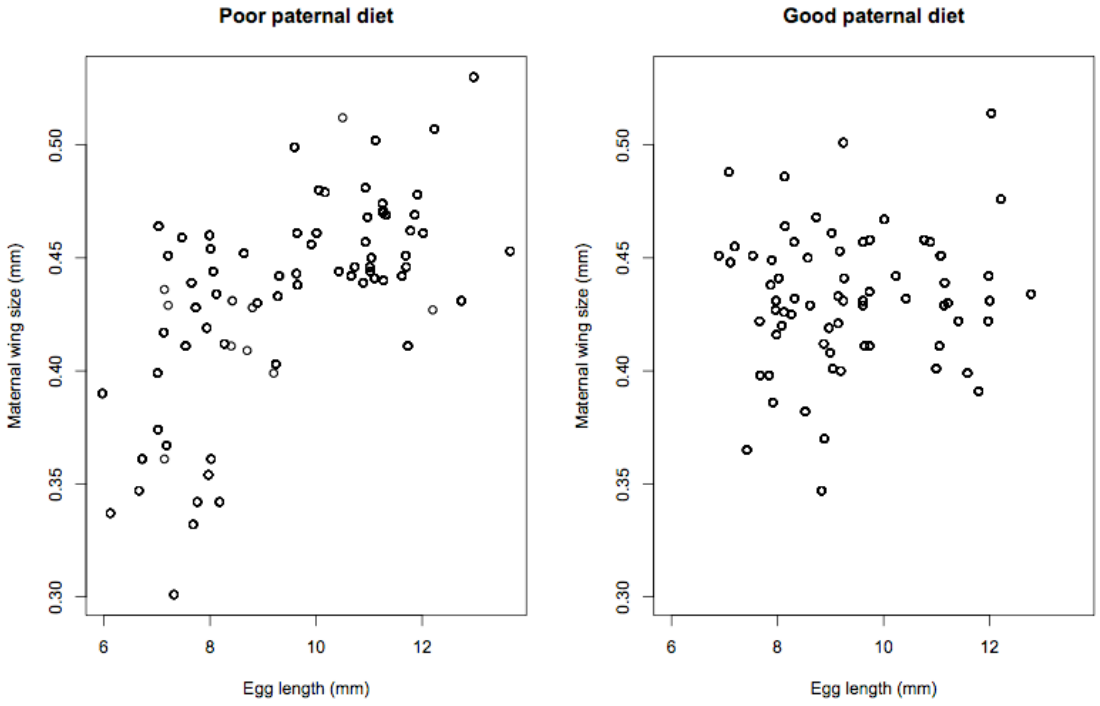

Figure 4.9: the effect of the interaction between maternal size and paternal diet on egg size.

4.5 Discussion

The aim of this study was to gain a better understanding of the parental effects of diet quality on offspring generations and how these effects may accumulate. My results show that diet quality has a profound effect on immune system activity, survival, egg size and fecundity, which is consistent with previous work, and increases understanding in this area by demonstrating that the diet given to both male and female parents affects

the immune function of their offspring. If the offspring themselves are given a good quality diet it goes some way to offset the negative parental effects.

4.5.1 Egg size

P. interpunctella eggs contain, among other things, lipids and proteins designed to oversee the development of the offspring (Bean et al. 1988). The eggs are stocked directly from the haemolymph of the female or from the nurse cells that surround it in the ovary (Telfer 2009). Therefore, the more resources a female has to invest in egg production the better stocked her eggs should be.

In our study, when a female was mated to a male that had received a good quality diet her egg size did not change according to her body size. However, when females were mated to a male that had received a poor quality diet egg size was correlated with female size, meaning that large females laid eggs of a comparable size to those laid by females mated with males given a good quality diet, but small females laid significantly smaller eggs. The smaller females, when mated to a male that had had a poor diet, may not have had the resources available to counter his lack of quality; poor diet males may not be transferring the resources to females during mating that the good quality diet males do, and a smaller female may not be able to compensate for this. Multiple mating in Lepidopterans increases the net seminal products received by a female, and this along with benefits from mating with virgin rather than experienced males, increases female fecundity (Torres-Vila and Jennions 2004; Torres-Vila et al. 2004). Male *Plodia interpunctella* also transfer extra resources to the female that are used for somatic

maintenance and egg production (Greenfield 1982). In our study the larger females may be attempting to compensate for a poorer quality mate by providing larger eggs to give her offspring a better start. Egg size may be related to offspring size (Sinervo & McEdward 1988; Rossiter 1991) and since there are many life history traits that correlate with offspring body size (Honěk 1993) a larger egg could boost the fitness of any offspring that are provided with ”low quality” genes or inherited environmental effects from a poorer quality father.

4.5.2 Immune function

Individuals raised on good quality food had a higher PO activity and haemocyte counts than those raised on poor quality food. Offspring haemocyte count responded strongly to parental diet, being highest in the offspring whose parents were both fed a good quality diet and lowest in those whose parents were both fed a poor quality diet. However, if one parent had a good quality diet and one a poor quality diet the haemocyte count was at an intermediate level regardless of which parent. Interestingly, these values are close to a midway point between the haemocyte count of those whose parents had a good diet and those whose parents had a poorer diet.

PO activity was highest in the offspring whose parents were both fed a good quality diet and lowest in those whose parents were both fed a poor quality diet, but a good quality offspring diet increased PO activity across all mating treatments. PO activity has a high heritability estimate (Cotter and Wilson 2002), which may explain why higher parental PO activities lead to higher offspring PO activity in our study. However, offspring diet interacted with parental PO activity, suggesting that although PO activity is largely

inherited it can be increased or decreased by the quality of the offspring diet. Over many generations this could lead to a population-wide increase or decrease via persistent environmental inheritance.

The effects of male diet on his offspring may be due to the effect male quality has on the quality of any nuptial gift, where a male provides a food item for the female to eat or transfers energy sources (Leopold 1976; Butlin et al. 1987) or minerals (Engerbretson and WMason 1980) to her from his accessory gland at mating. As these resources can be used by the female for her own needs or to help provision her eggs (Boggs 1995), a nuptial gift that is highly nutritious could be extremely beneficial to the offspring. Despite this, whether or not a nuptial gift benefits the offspring is not clear. Male bushcrickets will transfer a large spermatophore to the females at the time of mating that she then partly eats and this has been shown to affect the offspring survival rates in *Poecilimon veluchianus* (Reinhold 1998) and the number and size of the eggs laid by female katydids (Gwynne 1988; Gwynne and Brown 1994) and tettigoniids (Simmons 1990), but no effect has been found in *Leptophyes laticauda* (Vahed and Gilbert 1997) and *Poecilimon veluchianus* (Reinhold and Heller 1993). No effect has been found in the wartbiter *Decticus verrucivorus* (Wedell and Arak 1989) or the decorated cricket, *Gryllodes sigillatus,* (Will and Sakaluk 1994) even when the females were fed a low quality diet; however, in these two species the spermatophore is barely sufficient to fertilise all the females eggs which would leave little for consumption. Male *Drosophila* transfer a large number of seminal proteins to the female that affect many

traits (Wolfner 1997; Wolfner et al. 1997) such as ovulation, oogensis and sperm storage.

Haemocyte count varied between block, but there was no systematic pattern in the effect, and the effect sizes were not large. It is possible that diet effects could have occurred, as the diet was made up for each block separately. The first chapter of this thesis has shown that immune function can be very sensitive to small changes in environment, and, although the same recipe was followed each time, small variations could have caused minor fluctuations in haemocyte count between block. In future, a large amount of food could be made up to feed every block, so long as the food was thoroughly mixed to ensure an even distribution before each use and vitamin loss in storage could be prevented.

4.5.3 Survival

Individuals raised on good quality food survive better than those raised on poor quality food. However, the effects of a low quality diet on survival are reduced if at least one parent had a good quality diet, with a good quality father being more important to offspring survival than a good quality mother. This paternal effect on survival has also been shown in the hatching success of fish (Rideout et al. 2004), and larger male crickets are responsible for larger eggs and larger offspring (Weigensberg et al. 1998).

In our system, survival may be influenced by the quality of the nuptial gift given to the mother by the father and/or trans-generational epigenetic effects rather than direct genetic benefit. The parental generation can transfer information to their offspring via genomic imprinting that does not alter the actual DNA sequence (Mousseau & Fox 1998), and these effects can be transferred from the father and not just via the mother (Moret and Schmid-Hempel 2001; Sadd et al. 2005; Roth et al. 2009; Roth et al. 2010b, a). In crickets, males whose offspring have high viability will enhance the viability of offspring sired by a poorer quality male in a sperm competitive situation (Garcia-Gonzalez and Simmons 2007), which suggests that not all viability benefits are passed genetically.

This study demonstrates that a single environmental variable can cause significant parental effects on egg size, number, survival and immune system response via the processes discussed above, and that paternal effects are more important than initially thought. Potential sire effects will have to be considered in future work, as well as their interaction with maternal effects.

Chapter 5: The effects of inbreeding on immunity and life history

5.1 Introduction

It has been known for some time that inbreeding can produce negative effects on an organism (Darwin 1876, 1885; Hollingsworth and Maynard Smith 1955; Bowman and Falconer 1960). The chief cause of this "inbreeding depression" is thought to be the increased offspring homozygosity that arises from the mating of two related individuals. There are two possible mechanisms by which increased homozygosity can lead to a reduction in fitness. The first, overdominance, occurs when heterozygotes are superior to homozygotes at a given locus (also known as heterozygous advantage), and the second, partial dominance, occurs when an inbred line becomes fixed for recessive or partially recessive detrimental alleles, decreasing the fitness of the individuals in that line (Charlesworth and Willis 2009). Homozygosity of lethal alleles (those that produce a survival of less than 10% (Simmons and Crow 1977)) may be just as responsible for inbreeding depression as homozygosity of merely detrimental alleles (Simmons and Crow 1977; Sperlich et al. 1977). Those individuals with reduced fitness due to inbreeding will not have the same survival and reproductive success as those that maintain high fitness, thus eventually 'purging' deleterious alleles from an inbred population (Hollingsworth and Maynard Smith 1955; Falconer 1971; Bryant et al. 1990; Barrett and Charlesworth 1991). This 'fitness rebound' (Bryant et al. 1990) may be more important in wild, lab and domestic populations than previously thought, and it has been suggested that it could easily be overlooked (Saccheri et al. 1996) as viable small,

and often closed, populations of ordinarily outbred species do exist in labs all over the world as well as in the wild (Slatis 1959; O'Brien et al. 1985; Hoelzel et al. 1993; Lande and Ritland 1994). Templeton and Read (1984) put forward the idea that artificial selection and deliberate inbreeding could be used as a tool to purge deleterious recessives from a captive population, and under some circumstances selective inbreeding can be very important to animal breeders. However, before purging can occur, an initial reduction in population fitness must occur, which greatly increases the risk of extinction of that population. There is also a risk that deleterious alleles may become fixed in the population, possibly lowering the fitness of the population permanently (Whitlock 2000). Another consequence of inbreeding and increased homozygosity is a reduction in the genetic variability of the population, which could impact heavily on future adaptation and evolution.

Reductions in fitness in inbred populations have been measured in invertebrates (Hollingsworth and Maynard Smith 1955; Andreev et al. 1998; Gerloff et al. 2003b; Gerloff and Schmid-Hempel 2005; Calleri et al. 2006), vertebrates (Slatis 1959; Bowman and Falconer 1960; Hardiman et al. 1974; Reid et al. 2003) and plants (Willis 1993; Kittelson and Maron 2000; Ferrari et al. 2006; Delphia et al. 2009), and it is expected that the degree of inbreeding depression should correlate with the coefficient of inbreeding, *F*, if all loci additively combine (Falconer 1989). Falconer (1989) made the observation that inbreeding affects most greatly those traits directly related to fitness, such as fecundity, and less so those that do not have the same direct relationship with fitness, such as adult body weight, but he did not back up this observation with

empirical evidence. As mutations that directly affect fitness traits are likely to be extremely deleterious, there should be directional dominance (Lynch and Walsh 1998) since fitness characteristics usually have increased dominance variance (Crnokrak and Roff 1995), whereas mutations in genes not directly associated with fitness, such as those determining some morphological traits, are likely to have much less directional dominance.

Turning to immune reactivity, this can be affected by polymorphisms in genes responsible for coding key immune functions (Sackton et al. 2010). Therefore, inbreeding might be predicted to cause a direct reduction in immune function rather than as part of general pleiotrophic effects. We know comparatively little about inbreeding effects on the immune system of invertebrates, however, and virtually nothing of how any immune function response compares to the response of life history traits. The aim of this study was to address this by assessing the impact of multiple generations of full sibling inbreeding on phenoloxidase activity, egg number, egg size, survival and protein content of the haemolymph in *Plodia interpunctella*.

5.2 Methods

Three generations of full sibling matings were carried out in genetic lines from two distinct populations of *P. interpunctella*, one from the UK and one from Australia. The UK stock culture was started by crossing 3 strains obtained from throughout the UK, the first from an outbreeding colony maintained in the laboratory at the University of

Liverpool for 5 years, and before that at Imperial College at Silwood Park for 10 years, the second from the University of Sheffield and the third being a population kept at Queen Mary University of London. The resulting culture was maintained for 30 generations before the start of this experiment. The Australian population was obtained from the Plant Science department of the Primary Industries and Fisheries (part of the Department of Employment, Economic Development & Innovation) in Queensland, Australia. These *P. interpunctella* originated from wild collected animals from an infestation of organic mixed nuts from one merchant. These were crossed with a population from Western Australia and maintained in the lab in Queensland for 5 years. 10 inbred lines were maintained from the UK population and 10 from the Australian population along with 2 outbred control lines from each origin. All larvae were raised individually in Petri dishes (Sterilin) on the standard food mix (see methods section).

The larvae that hatched from 50 eggs from a single full-sib mating were raised individually in standard food medium. Once they had reached late 5^{th} instar 20 individuals were randomly selected and a haemolymph sample taken for immune and protein assays. All of these 20 larvae were allowed to pupate and eclose in individual 100ml pots and once hatched they were randomly allocated into brother and sister pairs. The eggs laid by the brother and sister pairs were counted every two days until the female died. One of these brother-sister pairs was chosen at random and their eggs were used to continue the line.

The two control lines from each origin were also raised individually as larvae, as with

the inbred group but they were grouped together as adults to mate as they would in a normal lab culture situation. Eggs were collected from randomly selected single females and those were photographed to allow wing measurements to be taken and used to continue with the line.

5.2.1 Analysis of data

For this study the unit of replication is the line, so mean values were calculated for line and the analysis carried out on these. General linear models were fitted to the immune function data with treatment (inbred or control), origin (UK or Australia) and generation plus all interactions as explanatory variables. Separate models were fitted for males and females. All data were log +1 transformed to allow proportional changes to be assessed, except for the protein analysis, in which the model was refitted with 1000 added to each data point to stabilise the error distribution after log transformation. Non-significant terms were removed following deletion tests (Crawley 2002) to leave a minimal adequate model. All analyses were performed using R version 2.8.1 for Macintosh.

5.3 Results

5.3.1 Protein levels of haemolymph

Although protein levels decreased with generations of inbreeding (females $F_{1,38} = 168.52$, $P < 0.001$; males $F_{1,38} = 135.67$, $P < 0.001$; Fig. 5.1) the UK and Australian lines responded slightly differently (origin effect on females $F_{1,38} = 60.47$, $P < 0.001$; origin effect on males $F_{1,38} = 33.13$, $P = 0.001$; Fig. 5.1). The Australian lines started

with much higher protein levels than the UK lines. After the first generation of inbreeding the protein levels of the Australian line fell to around that of the UK lines before any inbreeding events. The protein levels of the UK lines fell after the first generation of inbreeding to an intermediate level and remained at this level after the second generation of inbreeding, the Australian lines only reaching this level after two generations of inbreeding.

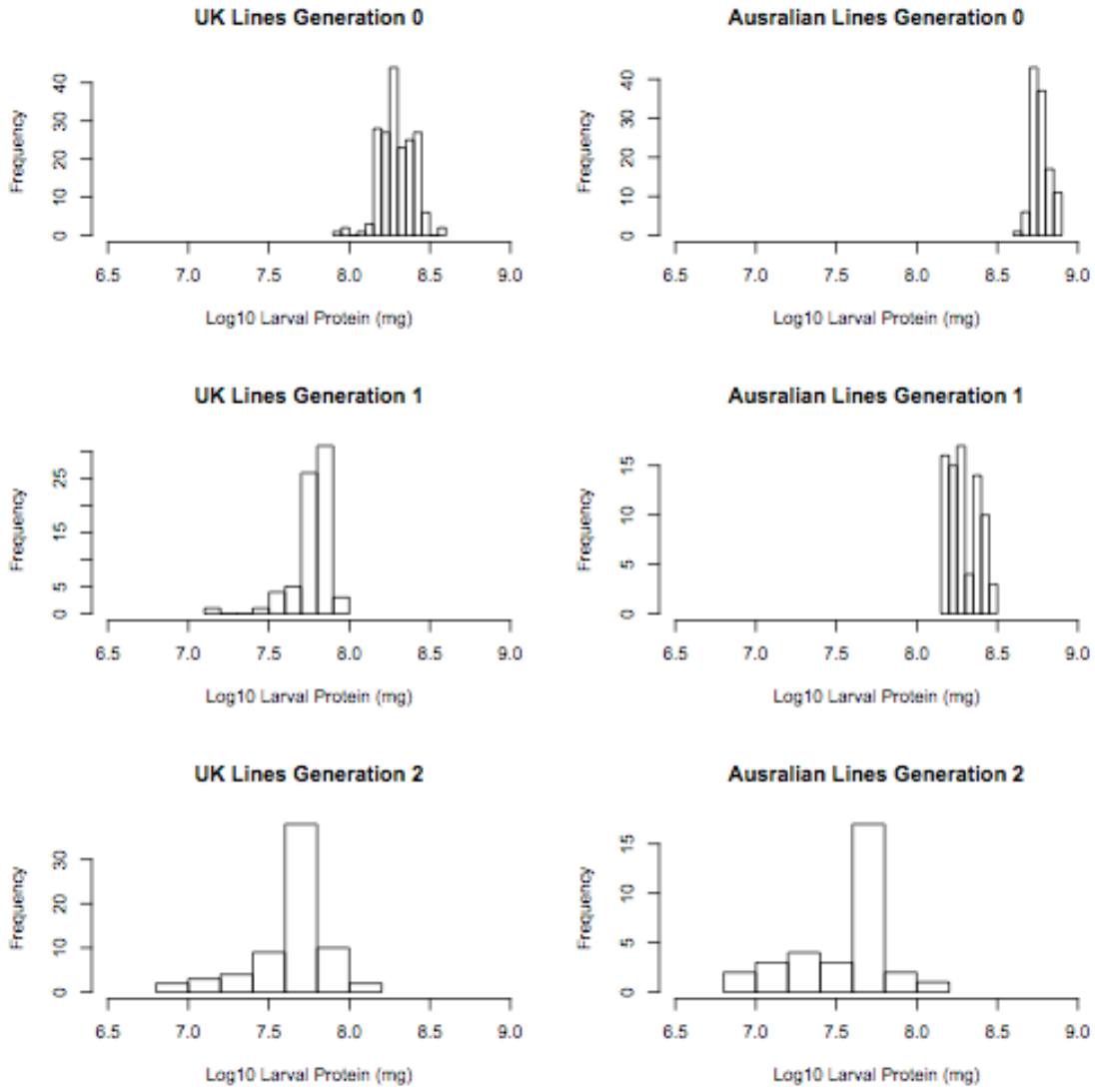

Figure 5.1: the effect of inbreeding on protein levels in the haemolymph of the UK and Australian lines.

5.3.2 PO activity

There was an interaction between generation and origin in males ($F_{1,37} = 12.17$, $P = 0.001$). In the Australian lines (Fig. 5.2) the males responded to inbreeding with a large decrease in PO activity after the first generation before remaining at this much lower level for a generation and then falling again after the 3rd generation. In contrast, the UK lines (Fig. 5.3) did not show much decrease at all and in two lines from the UK PO activity actually increased with inbreeding. In the Australian lines PO activity responded much more strongly to inbreeding in males than in females (females $F_{1,39} = 8.36$, $P = 0.006$; males $F_{1,37} = 15.92$, $P < 0.001$). In females the variance was increased more by inbreeding than in males. In some lines the inbred females had a higher PO activity than the control lines while some had a much lower activity, whereas in males the overall trend was for a slight decrease in PO activity.

5.3.3 Egg size

Egg length ($F_{1,35} = 81.52$, $P < 0.001$; Fig. 5.4) decreased with each generation of inbreeding. The first egg length model fitted produced a significant interaction term that suggested that the egg length of the UK and Australian lines reacted differently to inbreeding. However, once one Australian data point with very high leverage (Cook's distance>>1) was removed the interaction was no longer significant.

When the ratio between egg length and egg width was analysed there was no change in the shape of the eggs down the generations of inbreeding. However, there was a significant difference in the width to length ratio of eggs from the UK lines and those

from the Australian lines ($F_{1,35} = 14.26$, $P < 0.001$; Fig. 5.5). While eggs from either origin were the same width, Australian eggs were longer than the UK ($F_{1,35} = 58.97$, $P < 0.001$).

5.3.4 Egg number

Egg number, both those laid over 2 days ($F_{1,38} = 34.07$, $P < 0.001$; Fig. 5.6) and in total ($F_{1,39} = 32.74$, $P < 0.001$; Fig. 5.6), significantly decreased with every generation of inbreeding. There was no effect of origin on this trait.

5.3.5 Larval weight

Both female weight ($F_{1,38} = 58.88$, $P < 0.001$) and male weight ($F_{1,38} = 26.41$, $P < 0.001$) decreased with inbreeding. However, the Australian lines (Fig. 5.7) behaved differently from the UK lines (Fig. 5.7) (origin effect on females $F_{1,38} = 20.96$, $P < 0.001$; origin effect on males $F_{1,38} = 8.22$, $P = 0.007$). In the UK lines weight stabilised somewhat after the first generation of inbreeding, with little change other than a slight increase in variance after two generations of inbreeding. In the Australian lines the variance in weight between lines increased after one generation of inbreeding, with some lines decreasing in weight and some increasing in weight. After two generations of inbreeding the variance decreased again, but the females decreased in weight much more than the males.

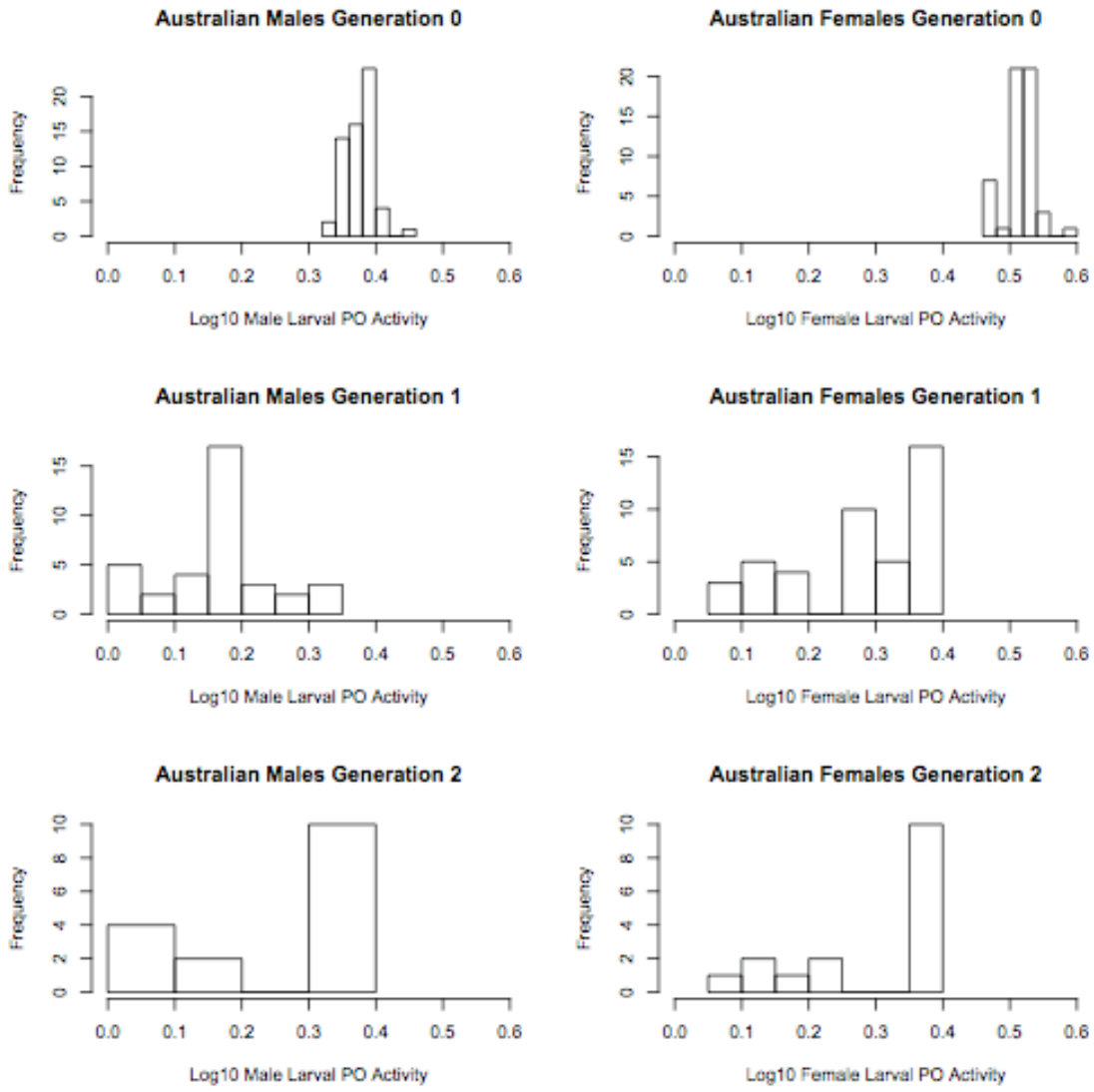

Figure 5.2: the effect of inbreeding on PO activity in males and females of the Australian lines.

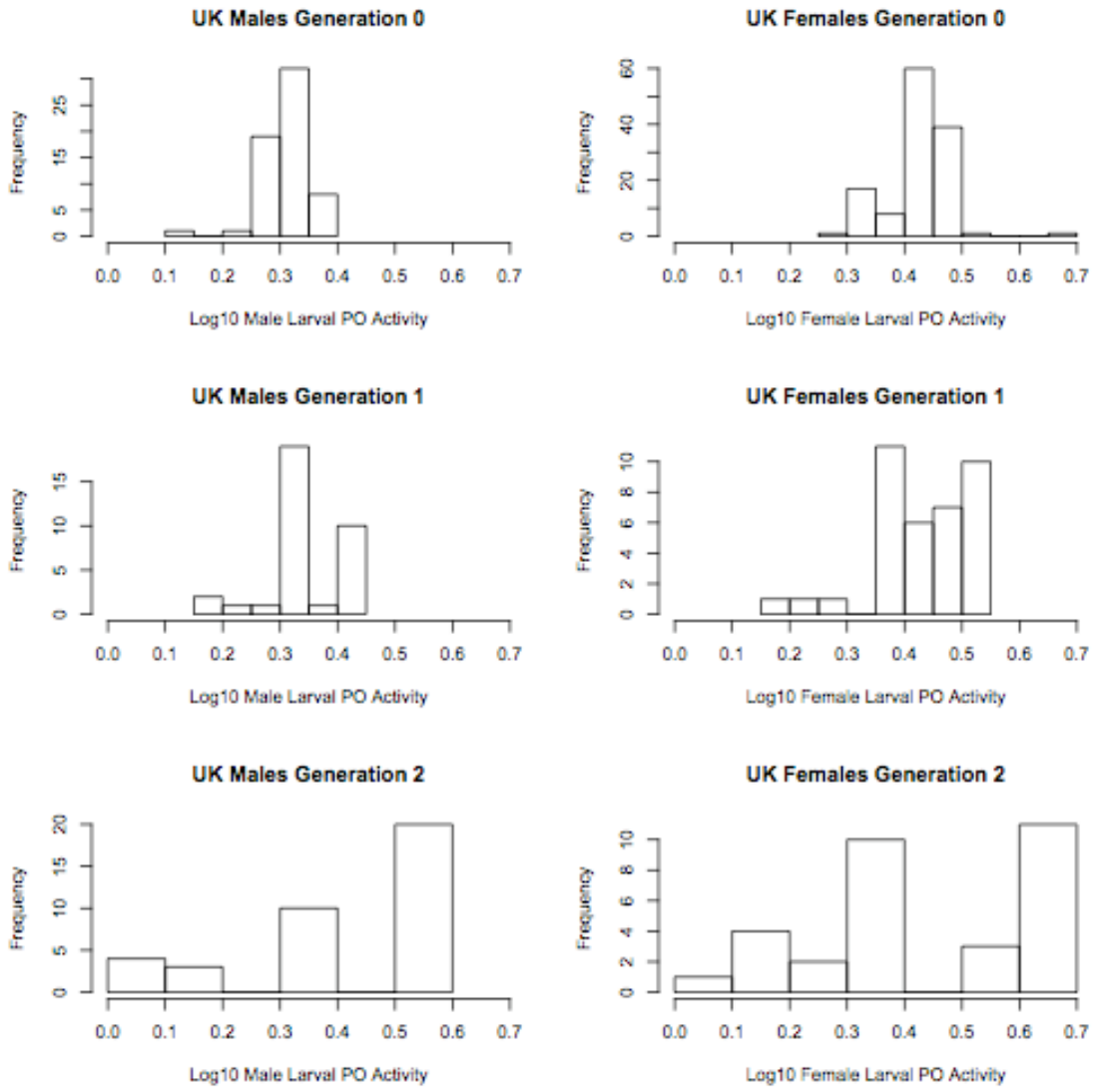

Figure 5.3: the effect of inbreeding on PO activity in males and females of the UK lines.

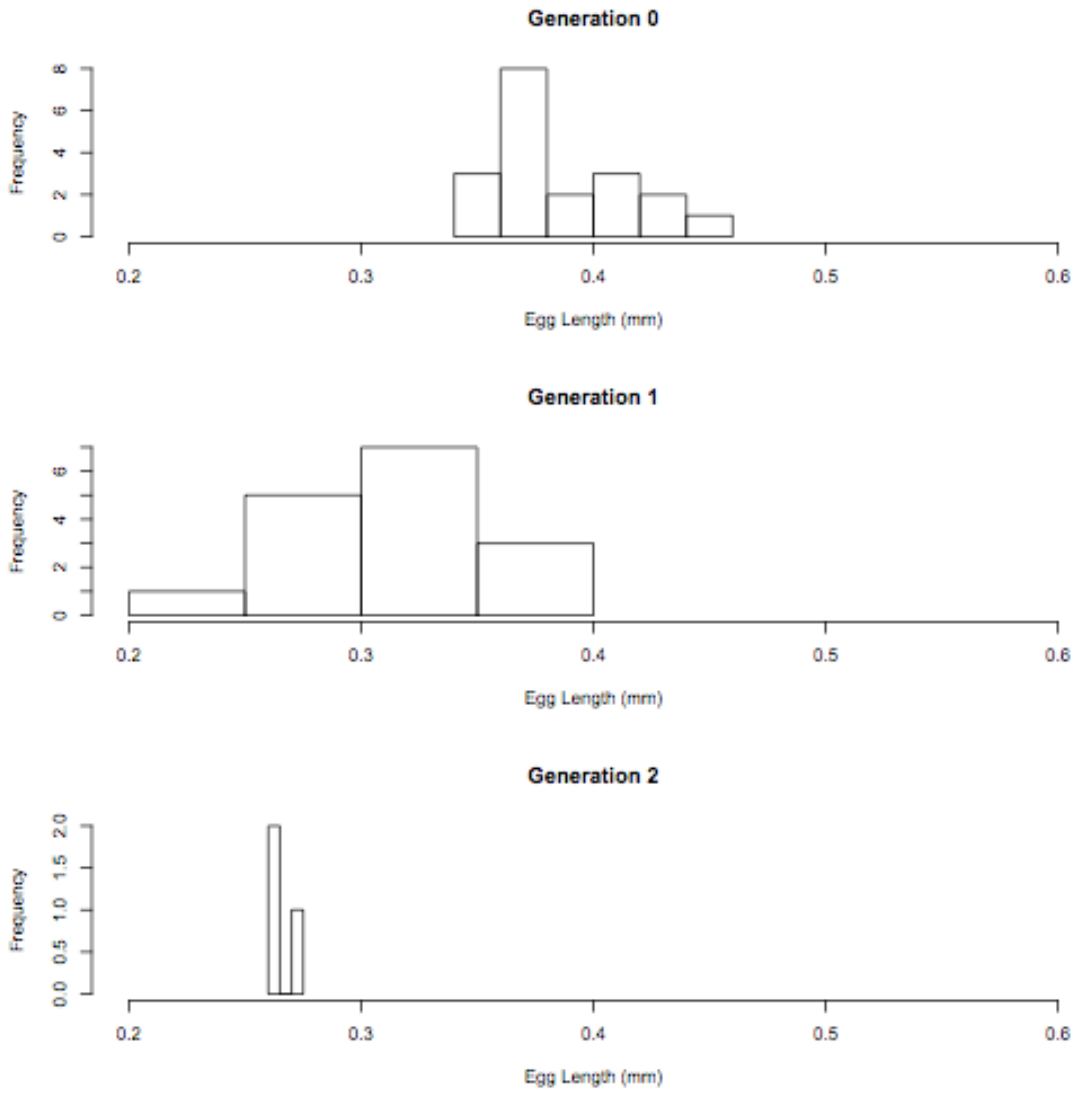

Fig. 5.4. The effect of inbreeding on egg size.

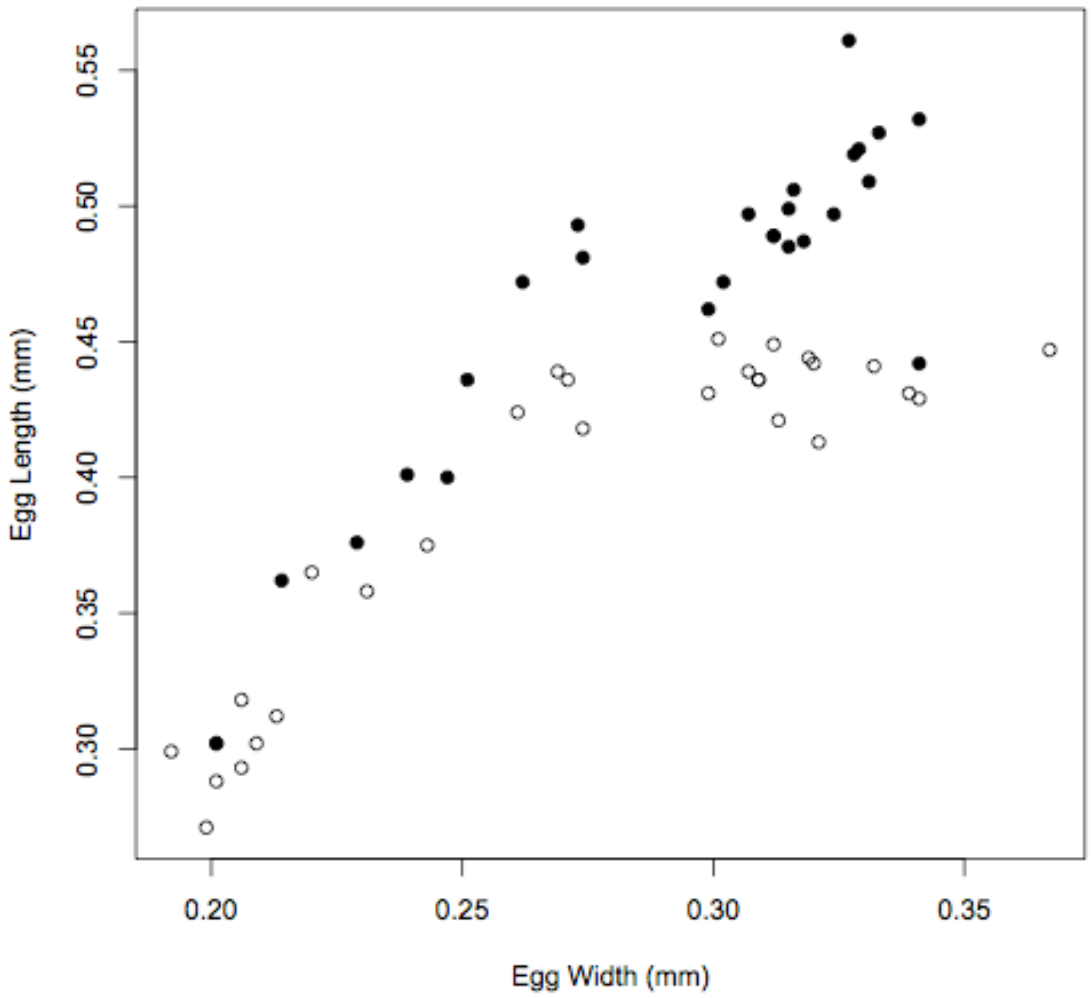

Fig. 5.5. Egg width to length ratio of the Australian and UK lines. The dark circles are the Australian lines.

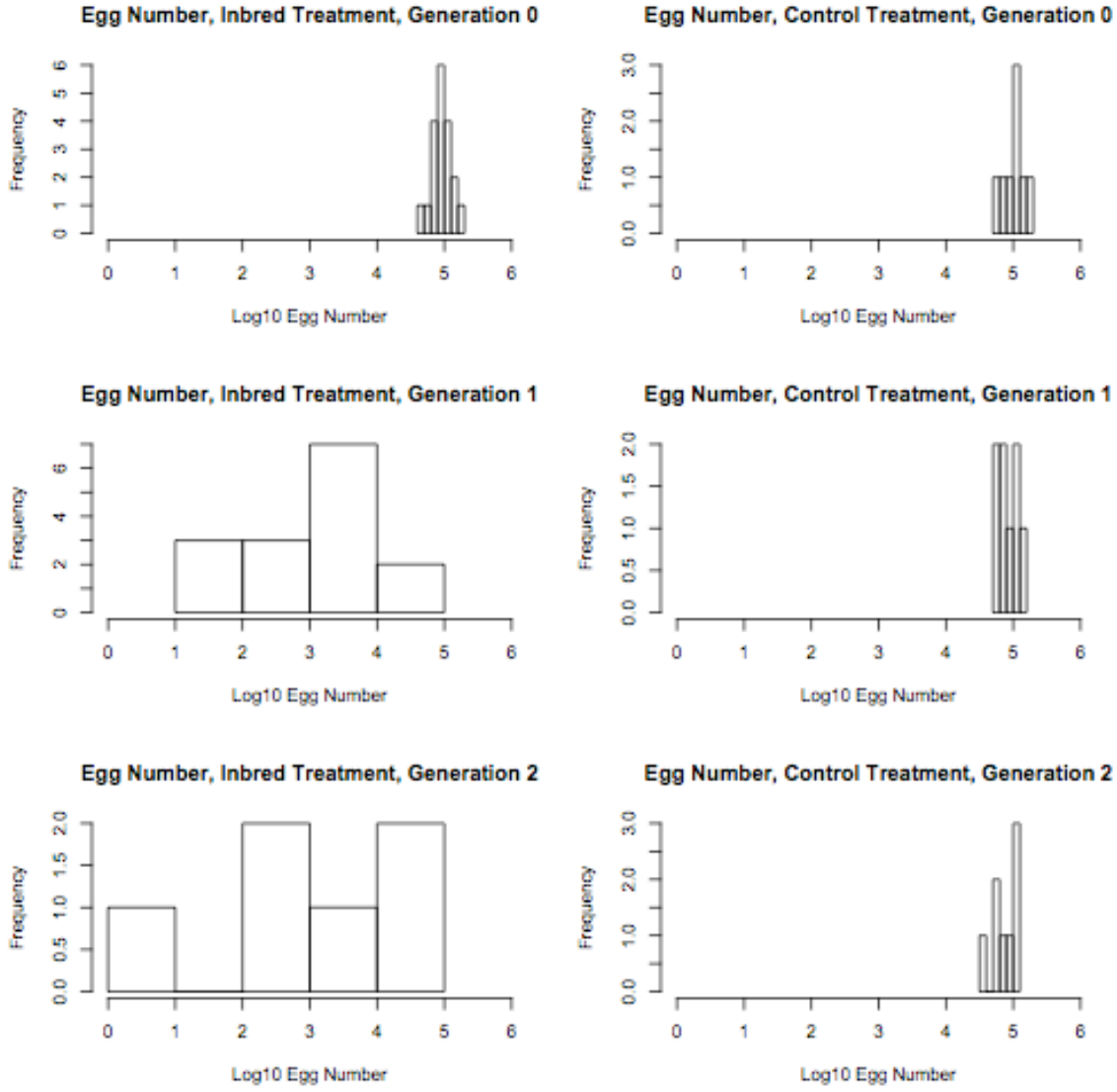

Fig. 5.6. Egg number with generation of inbreeding for the inbred and control lines.

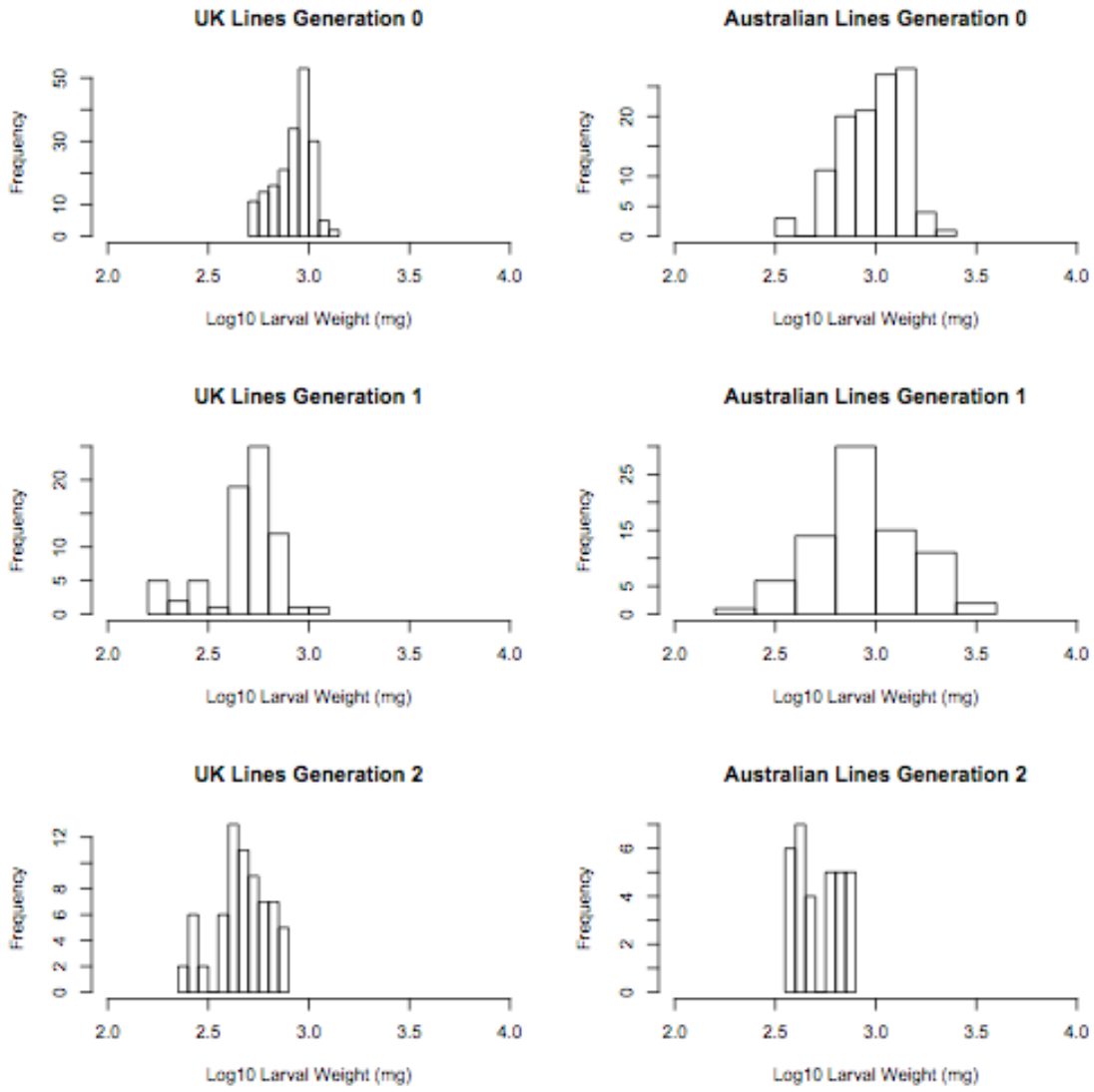

Fig. 5.7. Larval weight with generation of inbreeding for the UK and Australian lines.

5.3.6 Survival

Survival to the 5thnstar was analysed using a generalised linear model ~~model~~ fitted with a quasibinomial error term because of serious overdispersion in the data. There was no effect of generation or origin when this was fitted to the inbred lines only. When another model was fitted that included all the data and treatment (inbred or control) was included as a factor then treatment emerged as the only term remaining in a minimal adequate model ($F_{1,69} = 11.68$, $P = >0.001$). Inbreeding decreased survival in both the Australian and UK populations, but it did not continue to decrease down the generations of inbreeding (Fig. 5.9).

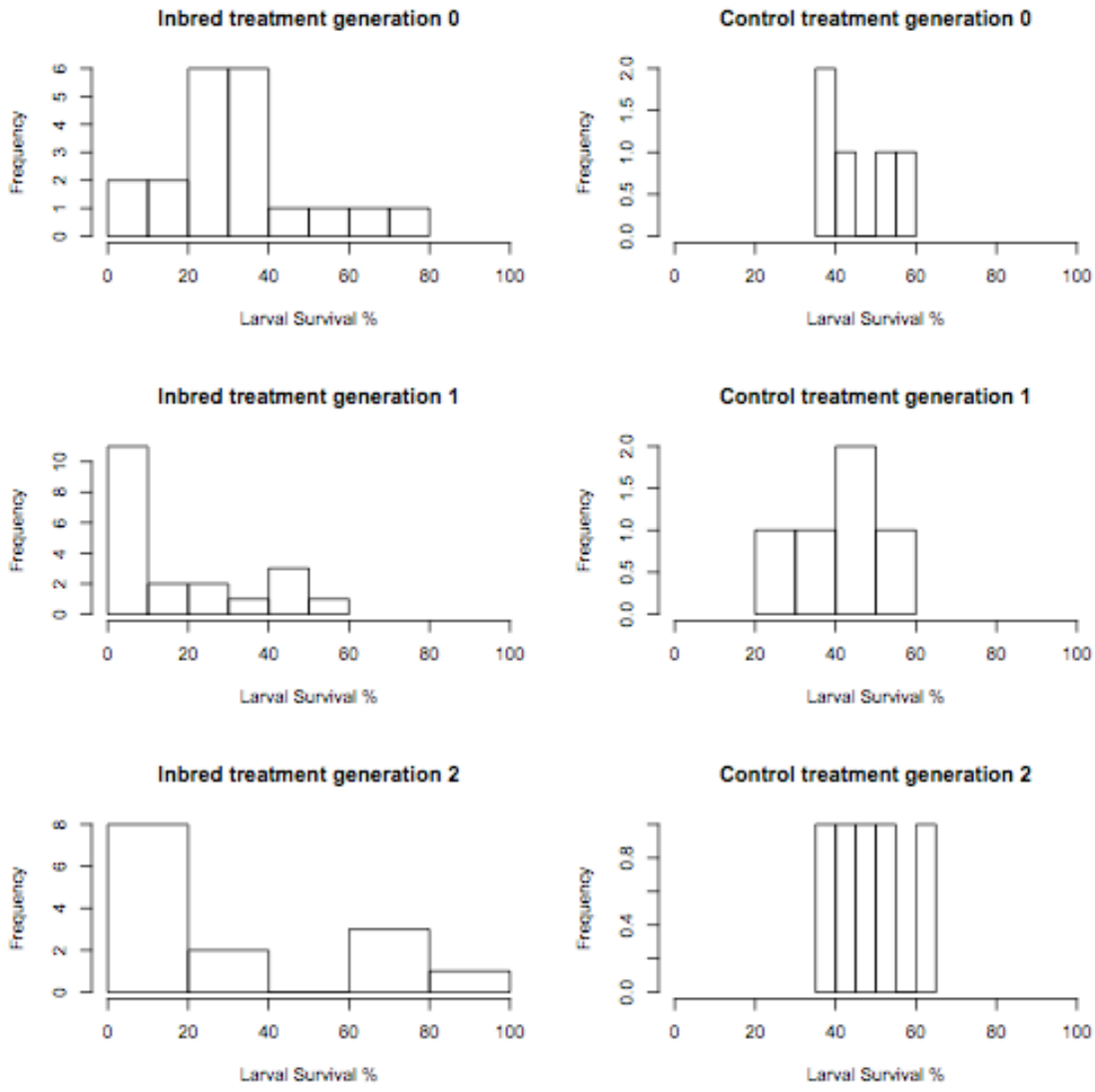

Fig. 5.8. Larval survival with generation of inbreeding for the inbred and control lines.

5.4 Discussion

Our results show that inbreeding has an effect on survival, egg size, fecundity, which is consistent with previous work. They also increase our understanding by demonstrating that phenoloxidase activity and protein levels of the haemolymph can be profoundly affected by inbreeding, but that the degree of inbreeding depression in both these variables is itself dependent on both origin and sex.

Proteins perform many important roles in the haemolymph of invertebrates. Haemocyanins carry oxygen around the body (Sackton et al. 2010), have considerable buffering potential towards carbon dioxide (Kerridge 1926; Andrews 1967) and may store amino acids and other organic nutrients (Schoffeniels 1976; Hagerman 1983). Apohaemocyanin does not contain copper, and so does not carry oxygen, but it allows rapid manufacture of haemocyanin (Horn and Kerr 1963) and stores nutrients for rapid use by other cells (Uglow 1969a, b, c). Fibrinogen and others are responsible for forming clots in response to injury (Bang 1963; Horn and Kerr 1963; Manwell and Baker 1963) and there are many more, such as hormones involved in moulting, ventilation and cardiac activity and proteins responsible for colouration and parts of the immune system, such as antibacterial proteins (Hildemann and Reddy 1973; Hildemann 1974; Kleinholz 1985; Trenczek and Bennich 1987; Morishima et al. 1992; Phipps et al. 1994) .

Our study showed that protein levels of individuals from both the UK and Australia

rapidly decreased with inbreeding. However, those from Australia had much higher protein levels to start with and the decrease over the first generation brought them in line with the original UK population. After the initial drop in protein levels after the first generation the UK population maintained their levels into the second generation, while the Australian lines only reached the same level after the second generation of inbreeding.

The reduction of protein levels in the haemolymph could have many consequences. We measured only total protein levels; therefore, it would be interesting to attempt to measure specific protein reductions to determine if some are traded off against others or if there is a general reduction in all proteins. As we cannot determine exactly which haemolymph proteins have decreased there may be a reduction in the immune function and wound healing of inbred individuals in ways we did not measure, such as antibacterial activity. Further work to determine whether other immune system traits and immune proteins are decreased would be extremely interesting.

Previous research looking at whether inbreeding affects immune function in invertebrates has been inconclusive. Some studies have found no reduction in innate immune function, such as antibacterial activity or encapsulation (Stevens et al. 1997; Gerloff et al. 2003a; Calleri II et al. 2006) while others have found a reduction in size and immune function in response to inbreeding (Rantala and Roff 2006). In this study, PO activity was used as an indicator of immune function; previously, research has shown that encapsulation of nylon is affected by inbreeding but no direct effect of

inbreeding on PO activity of the haemolymph has been shown (Rantala and Roff 2007). The UK populations did not show much decrease in PO activity at all when inbred; in fact, in two lines from the UK PO activity actually increased with inbreeding. This suggests that there may be multiple loci controlling PO activity in *P.interpunctella*, some that increase the activity and some that function to decrease it. It has been shown that high levels of PO activity can be detrimental to longevity and survival (Schwarzenbach and Ward 2006) and that the immune system can cause damage to the tissues of the invertebrate itself (Sadd and Siva-Jothy 2006): this autoreactivity has important life-history implications. If a high PO activity were detrimental to the organism the presence of controlling or limiting genes would be beneficial. As inbreeding reduces the genetic variability in a population, some individuals may inherit only genes that limit PO activity or only those that increase it, both of which could cause serious consequences or even mortality. The self-harm produced by too much PO activity may go some way to explaining why those lines with high PO activity did not succeed past two generations of inbreeding.

The PO activity of Australian males responded much more strongly to inbreeding than Australian females. Although sex differences in immune function are known to occur in vertebrates (Zuk and Stoehr 2002), the picture is less clear in invertebrates (Gray 1998; Kurtz et al. 2000; Adamo et al. 2001; Vainio et al. 2004; McKean and Nunney 2005; Rantala and Roff 2007) and in our study there were only sex differences in the population from Australia. Rantala and Roff (2007) demonstrated sex differences in the immune systems response to inbreeding in a more outbred species, and suggested that

species that naturally inbreed may be more resistant to the effects of inbreeding. One possible explanation for the difference in response to inbreeding between the two populations is that the UK lines were more inbred to start with, which might be explained by the Australian lines having been cultured in the lab for a much shorter time than the UK lines. As ancestral inbreeding can reduce inbreeding depression (Swindell and Bouzat 2006b, a), a population that has a history of past close inbreeding may show fewer effects of inbreeding than those who have come from a more outbred population. If the UK lines were more inbred at the start, some of the deleterious recessives may have already been purged from the population explaining why the factors measured stabilised after one generation of inbreeding. The increase in variance after inbreeding in the Australian lines may result from many of the original population being heterozygous at certain loci, and the resulting homozygosity after inbreeding produced offspring atypical of the parental generation.

Larval body size decreased with inbreeding in both populations, and small body size can reduce fecundity (Roff et al. 2002): this may explain why egg numbers, laid over two days and in total, significantly decreased with every generation of inbreeding in both populations. As survival of *P. interpunctella* larvae is already quite low, and this decreases a great deal further with inbreeding, low egg numbers might not cause only one genetic line to become extinct but an entire population. *P. interpunctella* are a typical r-selected species (Verhulst 1838), which grow fast, have a relatively small body size, a short generation time, are able widely to disperse their offspring, produce many offspring who each have a low probability of survival to adulthood and typically exploit

empty or less croded niches (Pianka 1972). If the number of eggs laid is greatly reduced so are the chances of any offspring reaching reproductive age, which could be disastrous for a species that relies so heavily on the sheer number of offspring produced per individual.

Egg size decreased with each generation of inbreeding, and since egg size may be related to offspring body size (Sinervo & McEdward 1988; Rossiter 1991) and many life history traits correlate with 8.9 offspring body size (Honěk 1993) it is possible that these smaller eggs could have lead to offspring with reduced fitness. As females stock the eggs with proteins, among other things, (Bean et al 1988) directly from the haemolymph or from the nurse cells that surround it in the ovary (Telfer 2009), the reduced levels of haemolymph protein after inbreeding may be affecting egg size directly and not just as a result of overall poor condition of the female.

The UK lines were more resistant to extinction, with most Australian lines being lost after the first generation of inbreeding and those lines that survived having fewer representatives while the UK lines maintained numbers fairly well right through to the second generation. As the lines that died out did not produce weight, protein or PO activity measurements following extinction, we can only speculate what they might have been, but it is notable that the lines that died out were the ones with very high and very low PO activity, suggesting that the middle ground is optimal in that case. The lines that survived the longest had larger larval weights ($t_{8.9}$= -4.96, p=<0.001) and produced eggs that were longer ($t_{12.5}$= -2.35, p=<0.005) and wider ($t_{14.3}$= -3.87,

p=<0.001) than the lines that became extinct most quickly, suggesting that general body condition and egg size play a major role in the survival of a population.

CHAPTER 6: General Discussion

This thesis has demonstrated just how plastic immune function can be, often due to very small environmental fluctuations or because of the environment experienced by an animal's parent. However, rather than just being a consequence of a costly immune system, plasticity may serve an adaptive purpose. Cases such as wing optimisation, where there are perhaps just one or very few best strategies, are very different from that of immune function, where the best strategy for individual success depends on the strategy of others in the population, pathogens and parasites (Maynard Smith 1982) and in an arms race responses have to be rapid and effective. To allow for immune strategies to change and adapt in response to environment fluctuations, pathogen evolution and competition with conspecifics there has to be room for plasticity in immune response.

In ecology it is important to understand how the environment influences immune function and life history traits and studies of model organisms such as *Plodia interpunctella*, the Indian meal moth can be of great use in understanding these issues. A review of the literature revealed that because maintaining and using an immune system is costly to the organism immune activity is expected to be condition-dependent and that many factors such as temperature, population density, diet and level of inbreeding are likely to have profound effects on immune system function and life history traits (Sheldon and Verhulst 1996; Kraaijeveld and Godfray 1997; Webster and Woolhouse 1999; Rigby and Jokela 2000; Kraaijeveld et al. 2001; Kraaijeveld et al. 2002)

Past studies of effects of environmental factors on immunity have focused mainly on one aspect of environmental fluctuation at a time. In chapter 3 I found that the interactions between various environmental factors are extremely important in deciding immune system strength or weakness in *P. interpunctella*: strong interactions between the three environmental variables led to the magnitude and in some cases the direction of the effects of most variables changing as the other environmental factors were altered.

Overall, food quality was the most important influence: larvae raised on a diet with higher levels of protein and sugar have substantially higher PO activity in every case and substantially higher haemocyte counts in all treatments except unheated:low density. When food quality was good the larvae showed "density dependent prophylaxis": raising their investment in immunity when population density is high. When food quality was poor, however, in most cases those larvae raised at high densities invested less in immunity. Temperature increases are often thought to lead to greater survival in pathogen infected ectotherms (Blumberg 1976; Kobayashi et al. 1981; Blumberg 1991; Geden 1997; Sigsgaard 2000; Frid and Myers 2002). In the case of PO activity an increased level of immune function was only evident when larvae were raised on good food, and in the low-density treatments a higher temperature led to reduced PO activity. A higher temperature led to higher haemocyte counts when density was high and food quality was good but when density was high and food quality poor the larvae had fewer haemocytes.

These results suggest that a good quality diet probably provided adequate resources to maintain or increase immune activity, while the poor quality diet resulted in immune activity being compromised. If resources are plentiful the larvae may have been able to invest more in their immune function at higher densities or temperatures, but the combined impact of high density and poor food has the opposite effect due, perhaps, to any potential for immune increase being limited by the lack of food quality or the result of other trade-offs due to density. In this case, an increased temperature only leads to higher immune reactivity under certain circumstances, and the two measured components of the immune system responded differently to temperature.

I have demonstrated the importance of considering interactions between different environmental variables when trying to understand the evolutionary ecology of immune responses. When an individual variable is studied in isolation important interactions and their effects may be overlooked. Such interactions could cause laboratory studies, where animals are kept in otherwise constant environments, to produce unreliable predictions of immune function in natural systems. Nonetheless, those experiments that consider single-factor responses do still allow us to gain some understanding of how the immune system reacts to environmental change. Multi-factorial experiments are often complex and the data difficult to interpret, and tackling one environmental variable at a time can provide important background information when looking at more than one variable and any interactions between them. The effect of food quality was consistent in all experiments suggesting that this has a very important influence on immune function regardless of other environmental variables. Furthermore, some environmental

influences cause consistent patterns across species, for example, density-dependant prophylaxis (Reeson et al. 1998 b; Wilson et al. 2001; Wilson et al. 2002)). However, these may only be due to lab experiments being carried out with other conditions controlled and near optimum. It is difficult to assess how much of this consistency is because of these highly controlled lab conditions and how much is due to there being a genuinely consistent and robust effect. At this time there is not enough empirical data to determine which is the case.

These results are especially important given ongoing concerns about anthropogenic climate change. Not only might the predicted increase in temperature change host-pathogen dynamics across natural environments, but as the environment changes food may become scarce which could cause clustering of species at high densities and/or effects of poor food quality on immune function and life history. An increase in pathogen virulence due to accelerated growth and increased transmission rates could produce dramatic effects on the ecosystem (Pounds 2001; Koelle et al. 2005), as could a reduction in virulence due to the parasite no longer being at optimum temperature or to improved host immune systems (Blanford et al. 2000), if this led to an increase in population densities of pest species it could produce important economic implications.

If climate change alters multiple environmental factors simultaneously, this could have profound effects on the evolution of an invertebrate species. Natural selection will act much more quickly on those organisms with faster generation times, and increasing temperature does decrease the time taken to reach maturity in many insect species

(Angilletta Jr et al. 2004). How this will affect the evolution of a species depends on the genetic architecture of the organism's response to more than one environmental variable. If there's a lot of genetic variability in the population controlling response to (for example) both temperature and food quality then the population will evolve fairly quickly and the change in climate won't affect the species so strongly. However, if there is only genetic variability in response to one variable then the population won't be able to evolve as fast and will be affected more strongly.

These strong interactions between environmental variables could be important in biological control. Ecological studies on the effects of the environment on the balance of host-parasite systems are frequently used to increase knowledge of biological control methods for pest species . The data presented in chapter 3 demonstrate that not only does the environment affect immune function, but also interactions between environmental variables can cause large variations in immune response. This suggests that the use of pathogens for pest control could have as many outcomes as there are combinations of environmental effects, casting doubt on their usefulness, especially if control is needed for long periods of time.

In chapter 4 I investigated the question of whether environmental effects on parents are seen in immune and life-history traits in their offspring. The diet quality experienced by an individual proved to cause significant effects on egg size, offspring survival and the immune system response of both parents and offspring: although a good quality diet for the offspring goes some way to offset these. While strong maternal effects on offspring

immunity might be expected the paternal effects that were clear throughout this experiment might be regarded as a surprise.

An interaction between paternal and maternal diet quality existed which affected egg size. Females laid larger eggs if they had been fed a good quality diet but were mated to a male that had been fed a poor quality diet and this suggests that the females might be trying to offset any negative effects on the offspring that a poorer quality male might have by increasing the resources she provides to each egg.

Individuals raised on good quality food had a higher PO activity and haemocyte counts than those raised on poor quality food, which is consistent with resource allocation theories and with the work done in chapter 3. Offspring haemocyte count and PO activity responded strongly to parental diet, both were highest in the offspring whose parents were both fed a good quality diet and lowest in those whose parents were both fed a poor quality diet, with an intermediate number being found in those offspring with one parent that had had a good quality diet and one a poorer quality diet regardless of which parent. However, a good quality offspring diet increased PO activity across all mating treatments.

The environment and individual experiences in very early life can have profound effects later (Linstrom 1999; Bateson et al 2004), and 'catch-up growth' has been shown in many different species that show indeterminate growth, where growth stops once the animals genetically pre-determined structure has completely formed (Bjorndal et al

2004; Alvarez & Nicieza 2005; Johnson & Bohlin 2006). In my study, some 'catch up' was seen in immune response and body size in those offspring whose parents were fed a poor quality diet when they themselves were fed a higher quality diet. There is very little data on 'catch up' growth in invertebrates, however, studies on vertebrates have shown that a number of costs are paid for any compensatory growth later in life (Metcalfe and Monaghan 2001; Hales and Ozanne 2003) and the results of this thesis suggest that immune activity could be permanently increased or decreased across a population by the quality of the diet experienced by breeding animals due to persistent environmental inheritance (Riska et al. 1985).

Finally, chapter 5 addressed the question of how inbreeding affects immune function. The *P. interpunctella* used were fairly resistant to the effects of inbreeding, with some genetic lines surviving repeated generations of brother to sister matings. However, there were some high costs to be paid in terms of larval size, egg size, survival and immune function.

Haemolymph protein levels of individuals from both the UK and Australia rapidly decreased with inbreeding, as did larval and egg size. After the initial drop in protein levels and larval size due to the first generation of inbreeding the UK population maintained their levels into the second generation. Although the Australian lines showed a decrease in overall protein and body size they increased variance from the mean after one generation of inbreeding, before the variance decreased with the amount of protein finally reaching the same levels as the UK lines after the second generation of

inbreeding and maintaining into the 3rd generation. This pattern suggests that the UK lines were more inbred to start with, which might be explained by the Australian lines having been cultured in the lab for a much shorter time than the UK lines. The smaller responses in the UK lines may be due to ancestral inbreeding producing genetic bottle necks that purged deleterious alleles from the population reducing the inbreeding depression seen in my study.

However, PO activity did not follow the same pattern as other features measured. The variance was much more increased by inbreeding, with some lines showing lower levels of PO activity while others showed higher levels than the control lines. As very high PO activity can actually cause self-harm and autoimmune effects, an individual with some alleles for increased PO activity and some to prevent levels becoming too high would have an advantage. Since inbreeding produces more homozygotes certain individuals, or whole genetic lines, may inherit two genes that limit PO activity or two that increase it, both of which could cause mortality and cause the increased variance from the mean that we saw. In unpredictable, highly variable environments genetic variance may be essential to a species survival (Lande and Shannon 1996). Inbreeding serves to increase the number of homozygotes in the population, with many generations of close inbreeding leading to animals that are highly similar genetically. The reduced number of alleles may lead to individuals, or whole inbred populations, with much less potential for immune plasticity which in turn could have serious knock on effects on population survival.

6.2 Future research and prospects

Factors such as sex differences and the changing environment can cause immune responses to vary widely across species and situations.

Much of the variability in vertebrate immune response will be due to the specificity of the acquired immune system. However, vertebrates are highly complex and invertebrates, with their relatively simple immune system, provide better analytical models for research into this area. Further research on the specificity of immune responses and immune memory in invertebrates may help to tease apart what is happening in the more complex immune systems of vertebrates. Cross-resistance and immunity trade-offs in invertebrates are interesting topics, for example, are individuals resistant to bacterial attack more or less susceptible to viral, fungal or parasitoid attack? Research into the level of specificity of an invertebrate immune response is to a pathogen type and whether this causes trade-offs within the immune system itself would produce interesting results.

How immune plasticity and function affects animals in more realistic situations should be addressed. I have gone some way to looking at this with my research into interactions between environmental variables, but more research in this field is needed. Separate experiments manipulating exact nutrients within the diet, rather than using a more general increase in diet quality, should tell us which parts of the diet have the biggest effect on immune function and life history. Also, more data on interactions

between environmental factors would be extremely useful, in *P. interpuntella* and other species with different life histories.

Whether factors such as inbreeding and diet quality do cause a measurable decrease in an individual's ability to fight disease, and not just an affect on some measure of immune response, needs to be ascertained. Further research that looks at an individual's ability to mount a response to a real pathogen would be extremely useful. We have various factors which affect two immune function traits and it would be extremely interesting to repeat some of the experiments in this thesis, but to infect a population of experimental insects with a disease and to induce the same immune challenge without the pathogen itself (for example, using a type of bacterium and LPS isolated from it). This should tell us how environmental factors affect the ability of an individual to fight off infection and/or mount some kind of immune response, and whether or not the differences in immune function parameters I have found here have real significance or are just arbitrary.

References

Abdel-Rahman, H. A., A. C. Hodson, and C. M. Christensen. 1968. Development of *Plodia interpunctella* (Hb.) (Lepidoptera, Phycitidae) on different varieties of corn at two levles of moisture. J Stored Prod Res 4:127-133.

Adamo, S. A. 1998. The specificity of behavioural fever in the cricket *Acheta domesticus*. J Parasitiol 84:529-533.

Adamo, S. A., M. Jensen, and M. Younger. 2001. Changes in lifetime immunocompetence in make and female *Gryllus texensis* (formally *G. integer*): trade-offs between immunity and reporoduction. Anim Behav 62:417-425.

Alford, R. A., and R. N. Harris. 1988. Effects of larval growth history on Anuran metamorphosis. Am Nat 131:91-106.

Anderson, R. M., and R. M. May. 1981. The population dynamics of microparasites and their invertebrate hosts. Phil. Trans. R. Soc. Lond. Series B-Biological Sciences 291:451-524.

Andreev, D., H. Breilid, L. Kirkendall, L. O. Brun, and R. H. Ffrench-Constant. 1998. Lack of nucleotide variability in a beetle pest with extreme inbreeding. Insect Mol Biol 7:197-200.

Andrews, P. 1967. Seasonal variation in the haemolymph composition in the crayfish *Orconectes limosus*. Zeitschrift vergl Physiol 57:7-43.

Angilletta Jr, M. J., T. D. Steury, and M. W. Sears. 2004. Temperature, growth rate and body size in ectotherms: fitting pieces of a life history puzzle. Integr Comp Biol 44:498-509.

Asada, N., N. Kawamoto, and H. Sezaki. 1999. Deleterious effect of null phenoloxidase mutation on the survival rate in *Drosophila melanogaster*. Dev Comp Immunol 23:535-543.

Asha, H., I. Nagy, G. Kovacs, D. Stetson, I. Ando, and C. R. Dearolf. 2003. Analysis of Ras-induced overproliferation in *Drosophila* hemocytes. Genetics 163:203-215.

Ashida, M., and P. T. Brey. 1995. Role of the integument in insect defense - pro phenol oxidase cascade in the cuticular matrix. Proc Natl Acad Sci USA 92:10698-10702.

Ashida, M., M. Ochiai, and H. Yoshida. 1986. Beta-1,3-glucan receptor and peptidoglycan receptor within prophenoloxidase activating system in insects. Dev Comp Immunol 10:623-623.

Ashida, M., and H. Yoshida. 1988. Limited proteolysis of prophenoloxidase during activation by microbial products in insect plasma and effect of phenoloxidase on electrophoretic mobilities of plasma-proteins. Insect Biochem 18:11-&.

Babin, A., B. Clotilde, and Y. Moret. 2010. Dietary supplementation with carotenoids improves immunity without increasing its cost in a crustacean. Am Nat 176:234-241.

Bang, F. B. 1963. Crustacean disease responses *in* A. J. Provenzano, ed. The Biology of Crustacea. Academy Press, New York.

Barnes, A. I., and M. T. Siva-Jothy. 2000. Density-dependent prophylaxis in the mealworm beetle *Tenebrio molitor* L-(Coleoptera : Tenebrionidae): cuticular melanization is an indicator of investment in immunity. Proc R Soc Lond B-Biological Sciences 267:177-182.

Barrett, S. C. H., and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352:522-524.

Bean, D. W., P. D. Shirk, and V. J. Brookes. 1988. Characterization of yolk proteins from the eggs of the Indian Meal Moth, *Plodia interpunctella*. Insect Biochem 18:199-&.

Bell, C. H. 1977. The sensitivity of larval *Plodia interpunctella* and *Ephestia elutella* to light during the photoperiodic induction of diapause. Physiol Entomol 2:167-172.

Bell, C. H. 1982. Observations on the intesity of diapause and cold tolerance in larvae from twelve populations and two reciprocal corsses of the Indian meal moth, *Plodia interpunctella*. Physiol Entomol 7:371-377.

Bell, C. H., C. R. Bowley, P. M. Cogan, and S. Sharma. 1979. Diapause in 23 populations of *Plodia interpunctella* (Hubner) (Lep., Pyralidae) from different parts of the world. Ecol Entomol 4:193-197.

Bell, C. H., and D. J. Walker. 1973. Diapause induction in *Esphestia elutella* (Hubner) and *Plodia interpunctella* (Hubner) (Lepidoptera, Pyralidae) with a dawn-dusk lighting system. J Stored Prod Res 9:149-158.

Bendelac, A., and D. T. Fearon. 1997. Innate immunity - innate pathways that control acquired immunity. Curr Opin Immunol 9:1-3.

Bernardo, J. 1996. Maternal effects in animal ecology. Am Zool 36:83-105.

Bernays, E. A., and R. F. Chapman. 1994. Host plant selection by phytophagous insects. Chapman & Hall, New York.

Bertram, D. F., and R. R. Strathmann. 1998. Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. Ecology 79:315-327.

Binnington, K. C., and F. M. Barrett. 1988. Ultrastructural localization of phenoloxidases in cuticle and haematopoietic tissue of the Blowfly *Lucilia cuprina*. Tissue Cell 20:405-419.

Blake, A., Y. Kotseridis, I. D. Brindle, D. Inglis, and G. J. Pickering. 2010. Effect of light and temperature on 3-alkyl-2-methoxypyrazine concentration and other impact odourants of Riesling and Cabernet Franc wine during bottle ageing. Food Chem 119:935-944.

Blandin, S., and E. A. Levashina. 2004. Thioester-containing proteins and insect immunity. Mol Immunol 40:903-908.

Blanford, S., A. F. Read, and M. B. Thomas. 2009. Thermal behaviour of *Anopheles stephensi* in response to infection with malaria and fungal entomopathogens. Malaria J 8:-.

Blanford, S., and M. B. Thomas. 1999a. Host thermal biology: the key to understanding insect-pathogen interactions and microbial pest control? Agr Forest Entomol 1:195-202.

Blanford, S., and M. B. Thomas. 1999b. Role of thermal biology in disease dynamics. Aspects Appl Biol 53:73-82.

Blanford, S., and M. B. Thomas. 2000. Thermal behavior of two acridid species: effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. Environ Entomol 29:1060-1069.

Blanford, S., M. B. Thomas, and J. Langewald. 1998. Behavioural fever in the Senegalese grasshopper, *Oedaleus senegalensis*, and its implications for biological control using pathogens. Ecol Entomol 23:9-14.

Blanford, S., M. B. Thomas, and J. Langewald. 2000. Thermal ecology of *Zonocerus variegatus*. Agr Forest Entomol 2:3-10.

Blanford, S., M. B. Thomas, C. Pugh, and J. K. Pell. 2003. Temperature checks the Red Queen? Resistance and virulence in a variable environment. Ecol Lett:2-5.

Bluhm, C. K., and P. A. Gowaty. 2004. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos.* Anim Behav 68:985-992.

Blumberg, D. 1976. Extreme temperatures reduce encapsulation of insect parasitoids in their insect hosts. Experientia 32:1396-1397.

Blumberg, D. 1991. Seasonal variations in the encapsulation of eggs of the encyrtid parasitoid, *Metaphycus stanleyi*, by the pyriform scale, *Protopulvinaria pyriformis*. Entomol Exp Appl:231-237.

Boersma, M., and J. Vijverberg. 1995. Synergistic effects of different food species on life history traits of *Daphnia galeata*. Hydrobiologia 307:109-115.

Boersma, M., and J. Vijverberg. 1996. Food effects on life history traits and seasonal dynamics of Ceriodaphnia pulchella. Freshwater Biol 35:25-34.

Boggs, C. L. 1995. Male nuptial gifts: penotypic consequences and evolutionary implications. Pp. 215-242 *in* S. R. Leather, and J. Hardie, eds. Insect Reproduction. CRC Press.

Boggs, C. L., and C. L. Ross. 1993. The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera, Nymphalidae). Ecology 74:433-441.

Bolton, M. 1991. Determinants of chick survival in the lesser Black-Backed Gull - relative contributions of egg size and parental quality. J Anim Ecol 60:949-960.

Boman, H. G., I. Nilsson, and B. Rasmuson. 1972. Inducible antibacterial defence system in *Drosophila*. Nature 237:232-&.

Bondari, K., R. L. Willham, and A. E. Freeman. 1978. Estimates of direct and maternal genetic correlations for pupa weight and family size of *Tribolium*. J Anim Sci 47:358-365.

Bonduriansky, R., and M. Head. 2007. Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera : Neriidae). J Evolution Biol 20:2379-2388.

Boorstein, S. M., and P. W. Ewald. 1987. Costs and benefits of Behavioural Fever in *Melanoplus sanguinipes* infected by *Nosema acridophagus*. Physiol Zool 60:586-595.

Bowman, J. C., and D. S. Falconer. 1960. Inbreeding depression and heterosis of litter size in mice. Genet Res 1:262-274.

Brey, P. T., W. J. Lee, M. Yamakawa, Y. Koizumi, S. Perrot, M. Francois, and M. Ashida. 1993. Role of the integument in insect immunity - epicuticular abrasion and induction of Cecropin synthesis in cuticular epithelial cells. Proc Natl Acad Sci USA 90:6275-6279.

Brockelman, W. Y. 1975. Competition, the fitness of offspring, and optimal clutch size. Am Nat 109.

Bryant, E. H., L. M. Meffert, and S. A. Mccommas. 1990. Fitness rebound in serially bottlenecked populations of the House Fly. Am Nat 136:542-549.

Bulet, P. 1999. Drosophila antimicrobial peptides. M S-Med Sci 15:23-29.

Bulet, P., S. Cociancich, J. L. Dimarcq, J. Lambert, J. M. Reichhart, D. Hoffmann, C. Hetru, and J. A. Hoffmann. 1991. Insect immunity - isolation from a coleopteran insect of a novel inducible antibacterial peptide and of new members of the insect defensin family. J Biol Chem 266:24520-24525.

Bulet, P., C. Hetru, J. L. Dimarcq, and D. Hoffmann. 1999. Antimicrobial peptides in insects; structure and function. Dev Comp Immunol 23:329-344.

Bulet, P., and R. Stocklin. 2005. Insect antimicrobial peptides: Structures, properties and gene regulation. Protein Peptide Lett 12:3-11.

Bull, J. O. 1970. Deal with insect pests this way. Food Manuf 45:46-&.

Burley, N. 1988. The differential-allocation hypothesis: an experimental test. Am Nat 132:611-628.

Butlin, R. K., C. W. Woodhatch, and G. M. Hewitt. 1987. Male spermatophore investment increases female fecundity in a grasshopper. Evolution 41:221-225.

Calleri, D. V., E. M. Reid, R. B. Rosengaus, E. L. Vargo, and J. F. A. Traniello. 2006. Inbreeding and disease resistance in a social insect: effects of heterozygosity on immnnocompetence in the termite *Zootermopsis angusticollis*. Proc R Soc Lond B-Biological Sciences 273:2633-2640.

Calleri II, D. V., E. McGrail Reid, R. S. Resengaus, E. L. Vargo, and J. F. A. Traniello. 2006. Inbreeding and disease resistance in a social insect: effect of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis.* Proc R Soc Lond B - Biological Sciences:2633-2640.

Campero, M., M. De Block, F. Ollevier, and R. Stoks. 2008. Correcting the short-term effect of food deprivation in a damselfly: mechanisms and costs. J Anim Ecol 77:66-73.

Carey, J. R., P. Liedo, L. Harshman, X. Liu, H. G. Muller, L. Partridge, and J. L. Wang. 2002. Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. Funct Ecol 16:313-325.

Carius, H. J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. Evolution 55:1136-1145.

Carlsson, A., P. Engstrom, E. T. Palva, and H. Bennich. 1991. Attacin, an antibacterial protein from *Hyalophora cecropia*, inhibits synthesis of outer membrane proteins in *Escherichia coli* by interfering with Omp gene-transcription. Infect Immun 59:3040-3045.

Carruthers, R. I., T. S. Larkin, H. Firstence, and Z. Feng. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. Ecology 73:190-204.

Chalk, R., H. Townson, S. Natori, H. Desmond, and P. J. Ham. 1994. Purification of an insect defensin from the mosquito, *Aedes aegypti*. Insect Biochem Molec 24:403-410.

Charalambidis, N. D., C. G. Zervas, M. Lambropoulou, P. G. Katsoris, and V. J. Marmaras. 1995. Lipopolysaccharide stimulated exocytosis of non-self recognition protein from insect hemocytes depend on protein tyrosine phosphorylation. Eur J Cell Biol 67:32-41.

Charlesworth, D., and J. H. Willis. 2009. Fundamental concepts in Genetics: the genetics of inbreeding depression. Nat Rev Genet 10:783-796.

Christensen, B. M., and D. W. Severs en. 1993. Biochemical and molecular basis of mosquito susceptibility to *Plasmodium* and filaroid nematodes. Pp. 245-266 *in* N. E. Beckage, S. N. Thompson, and B. A. Federici, eds. Parasites and Pathogens of Insects. Academic, San Diego.

Cociancich, S., A. Ghazi, C. Hetru, J. A. Hoffmann, and L. Letellier. 1993. Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in *Micrococcus luteus*. J Biol Chem 268:19239-19245.

Cooper, E. L. 1968. Transplantation immunity in annelids I. Rejection of xenografts exchanged between *Lumbricus terrestris* and *Eisenia foetida*. Transplantation 6:322-327.

Cooper, E. L., and L. M. Rubilotta. 1969. Allograft rejection in *Eisenia foetida*. Transplantation 8:220-223.

Costa, S. C. P., C. Ribeiro, P. A. Girard, R. Zumbihl, and M. Brehelin. 2005. Modes of phagocytosis of Gram-positive and Gram-negative bacteria by *Spodoptera littoralis* granular haemocytes. J Insect Physiol 51:39-46.

Cotter, S. C., R. S. Hails, J. S. Cory, and K. Wilson. 2004. Density-dependant prophylaxis and condition dependant immune function in Lepidopteran larvae: a multivariate approach. J Anim Ecol 73:283-293.

Cotter, S. C., S. J. Simpson, D. Raubenheimer, and K. Wilson. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. Funct Ecol In press.

Cotter, S. C., and K. Wilson. 2002. Heritability of immune function in the caterpillar *Spodoptera littoralis*. Heredity 88:229-234.

Cotton, R. T. 1963. Pests of stored grain and grain products. Burgess Publishing Co., Minnesota.

Cox, P. D., and C. H. Bell. 1991. Biology and ecology of moth pests of stored foods. Pp. 181-193 *in* J. R. Gorham, ed. Ecology and Management of Food-Industry Pests, FDA Technical Bulletin 4. Association of Official Analytical Chemists, Arlington, Virginia.

Crawley, M. J. 2002. Statistical Computing: An Introduction to Data Analysis Using S-Plus. WileyBlackwell, Chichester, UK.

Crnokrak, P., and D. Roff. 1995. Dominance variance: associations with selection and fitness. Heredity 75:530-540.

Cunningham, E. J. A., and A. F. Russell. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:74-77.

Darwin, C. R. 1876. The Effects of Cross and Self-fertilisation in the Vegetable Kingdom. Hard Press.

Darwin, C. R. 1885. Variation of Animals and Plants Under Domestication. J. Murray, London.

Davies, D. H., and M. T. Siva-Jothy. 1991. Encapsulation in insects: polydnaviruses and encapsulation-promoting factors. Pp. 120-132 *in* A. P. Gupta, ed. Immunity of Insects and Other Arthropods. CRC, Boca Raton.

De Gregorio, E., P. T. Spellman, G. M. Rubin, and B. Lemaitre. 2001. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. Proc Natl Acad Sci USA 98:12590-12595.

Delphia, C. M., J. R. Rohr, A. G. Stephenson, C. M. De Moraes, and M. C. Mescher. 2009. Effects of genetic variation and inbreeding on volatile production in a field population of Horsenettle. Int J Plant Sci 170:12-20.

Dickinson, L., V. W. Russell, and P. E. Dunn. 1988. A family of bacteria-regulated cecropin D-like peptides from *Manduca sexta*. J Biol Chem 263:19424-19429.

Dimarcq, J. L., D. Hoffmann, M. Meister, P. Bulet, R. Lanot, J. M. Reichhart, and J. A. Hoffmann. 1994. Characterization and transcriptional profiles of a *Drosophila* gene encoding an insect defensin - a study in insect immunity. Eur J Biochem 221:201-209.

Dimarcq, J. L., E. Keppi, B. Dunbar, J. Lambert, J. M. Reichhart, D. Hoffmann, S. M. Rankine, J. E. Fothergill, and J. A. Hoffmann. 1988. Insect immunity - purification and characterization of a family of novel inducible antibacterial proteins from immunized larvae of the Dipteran *Phormia terranovae* and complete amino acid sequence of the predominant member, Diptericin-A. Eur J Biochem 171:17-22.

Dimarcq, J. L., E. Keppi, J. Lambert, D. Zachary, and D. Hoffmann. 1986. Diptericin-a, a novel antibacterial peptide induced by immunization or injury in larvae of the Dipteran insect *Phormia terranovae*. Dev Comp Immunol 10:626-626.

Doering, T. L., J. D. Nosanchuk, W. K. Roberts, and A. Casadevall. 1999. Melanin as a potential cryptococcal defence against microbicidal proteins. Med Mycol 37:175-181.

Dunn, P. E., T. Bohnert, and V. W. Russell. 1994. Midgut antibacterial defenses of Manduca seta following infection and during metamorphosis. Pp. 105-111 *in* J. A. Hoffman, C. A. J. Janeway, and S. Natori, eds. Phylogenetic Persepectives in Immunity: The Insect Host Defese. CRC Press, Boca Raton.

Dunn, P. E., W. Dai, M. R. Kanost, and C. X. Geng. 1985. Soluble peptidoglycan fragments stimulate antibacterial protein synthesis by fat body from larvae of *Manduca sexta.* Dev Comp Immunol 9:559-568.

Duvic, B., and K. Soderhall. 1992. Purification and partial characterization of a beta-1,3-glucan binding protein membrane receptor from blood cells of the Crayfish *Pacifastacus leniusculus*. Eur J Biochem 207:223-228.

Ehlers, D., B. Zosel, W. Mohrig, E. Kauschke, and M. Ehlers. 1992. Comparison of in vivo and in vitro Phagocytosis in *Galleria mellonella* L. Parasitol Res 78:354-359.

Emlet, R. B., and O. HoeghGuldberg. 1997. Effects of egg size on postlarval performance: experimental evidence from a sea urchin. Evolution 51:141-152.

Engerbretson, J. A., and W. H. WMason. 1980. Transfer of Zn at Mating in *Heliothis virescens*. Environ Entomol 9:119-121.

Eslin, P., and G. Prévost. 1996. Variation in *Drosophila* concentration of haemocytes associated with different ability to encapsulate *Asobara tabida* larval parasitoid. J Insect Physiol 42:549-555.

Fabrick, J. A., J. E. Baker, and M. R. Kanost. 2003. cDNA cloning, purification, properties, and function of a beta-1,3-glucan recognition protein from a pyralid moth, *Plodia interpunctella*. Insect Biochem Molec 33:579-594.

Falconer, D. S. 1971. Improvement of litter size in a strain of mice at a selection limit. Genet Res 17:215-&.

Falconer, D. S. 1989. Introduction to Quantitative Genetics. Longman, New York.

Faulhaber, L. M., and R. D. Karp. 1992. A diphasic immune response against bacteria in the American cockroach. Immunology 75:378-381.

Fearon, D. T. 1997a. Innate and acquired immunity. Nature 388:323-323.

Fearon, D. T. 1997b. Seeking wisdom in innate immunity. Nature 388:323-324.

Feder, D., C. B. Mello, E. S. Garcia, and P. Azambuja. 1997. Immune reactions in *Rhodnius prolixus*: influence of nutrition and ecdysone. J Insect Physiol 43:513-519.

Fehlbaum, P., P. Bulet, S. Chernysh, J. P. Briand, J. P. Roussel, L. Letellier, C. Hetru, and J. A. Hoffmann. 1996. Structure-activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides. Proc Natl Acad Sci USA 93:1221-1225.

Fellowes, M. D. E., and H. C. J. Godfray. 2000. The evolutionary ecology of resistance to parasitoids by *Drosophila*. Heredity 84:1-8.

Ferrari, M. J., A. G. Stephenson, M. C. Mescher, and C. M. De Moraes. 2006. Inbreeding effects on blossom volatiles in *Cucurbita pepdo* subsp *texana* (Cucurbitaceae). Am J Bot 93:1768-1774.

Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol 45:341-369.

Fox, C. W., and U. M. Savalli. 2000. Maternal effects mediate host expansion in a seed-feeding beetle. Ecology 81:3-7.

Frid, L., and J. H. Myers. 2002. Thermal ecology of western tent caterpillars, *Malacosoma californnicum pluviale*, and infection by nucleopolyhedrovirus. Ecol Entomol 27:665-673.

Gage, M. J. G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. Proc R Soc Lond B-Biological Sciences 261:25-30.

Garcia-Gonzalez, F., and L. W. Simmons. 2007. Paternal indirect genetic effects on offspring viability and the benefits of polyandry. Curr Biol 17:32-36.

Garner, T. W. J., J. M. Rowcliffe, and M. C. Fisher. 2010. Climate change, chytridiomycosis or condition: an experimental test of amphibian survival. Global Change Biol 16:1354-1013.

Gazit, E., W. J. Lee, P. T. Brey, and Y. C. Shai. 1994. Mode of Action of the Antibacterial Cecropin B2 - a Spectrofluorometric Study. Biochemistry-Us 33:10681-10692.

Geden, C. J. 1997. Evaluation of *Paraiotonchium muscadomesticae* (Nematoda: Tylenchida: Iotonchiidae), a potential biological control agent of the housefly (Diptera: Muscidae). Biol Control 10:42-47.

Gerloff, C. U., B. K. Ottmer, and P. Schmid-Hempel. 2003a. Efects of imbreeding on immune response and body size in a social insect, *Bombus terrestris*. Funct Ecol 17:582-589.

Gerloff, C. U., B. K. Ottmer, and P. Schmid-Hempel. 2003b. Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. Funct Ecol 17:582-589.

Gerloff, C. U., and P. Schmid-Hempel. 2005. Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera : Apidae). Oikos 111:67-80.

Gilchrist, G. W., and R. B. Huey. 2001. Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. Evolution 55:209-214.

Gillespie, J. P., M. R. Kanost, and T. Trenczek. 1997. Biological mediators of insect immunity. Annu Rev Entomol 42:611-643.

Götz, P. 1986. Encapsulation in arthropods. Pp. 153-170 *in* M. Brehelin, ed. Immunity in Invertebrates: Cells, Molecules and Defense Reactions. Springer Verlag, Berlin.

Goulson, D. 1994. Determination of larval melanisation in the cabbage moth, *Mamestra brassicae*, and the role of melanism in thermoregulation. Heredity 73:471-479.

Goulson, D., R. S. Hails, T. Williams, M. L. Hirst, S. D. Vasconcelos, B. M. Green, T. H. Carty, and J. S. Cory. 1995. Transmission dynamics of a virus in a stage-structured insect population. Ecology 76:392-401.

Gowaty, P. A. 2008. Reproductive compensation. J Evol Biol 21:1189-1200.

Gray, D. A. 1998. Sex differences in susceptibility of house crickets, *Acheta domesticus*, to experimental infection with *Serratia liquefaciens*. J Invertebr Pathol 71:288-289.

Greenfield, M. D. 1982. The question of paternal investment in lepidoptera: male-contributed proteins in *Plodia interpunctella*. Int J Inver Rep Dev 5:323-330.

Grindstaff, J. L., E. D. Brodie, and E. D. Ketterson. 2003. Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. Proc R Soc Lond B Bio 270:2309-2319.

Grindstaff, J. L., D. Hasselquist, J. A. Nilsson, M. I. Sandell, and M. Stjernman. 2002. Maternal effects of antigen challenge on offspring growth and immunity in wild birds. Integr Comp Biol 42:1237-1237.

Gunn, A. 1998. The determination of larval phase coloration in the African armyworm, *Spodoptera exempta* and its consequences for thermoregulation and protection from UV light. Entomol Exp Appl 86:125-133.

Gwynne, D. T. 1988. Courtship feeding and the fitness of female Katydids (Orthoptera, Tettigoniidae). Evolution 42:545-555.

Gwynne, D. T., and W. D. Brown. 1994. Mate feeding, offspring investment, and sexual differences in Katydids (Orthoptera, Tettigoniidae). Behav Ecol 5:267-272.

Hagerman, L. 1983. Haemocyanin concentration of juvenile lobsters (Homarus gammarus) in relation to moulting cycle and feeding conditions. Mar Biol 77:11-19.

Hajek, A. E., T. S. Larkin, R. I. Carruthers, and R. S. Soper. 1993. Modeling the dynamics of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations. Environ Entomol 22:1172-1187.

Hales, C. N., and S. E. Ozanne. 2003. The dangerous road of catch-up growth. J Physiol 547:5-10.

Hanrahan, J. P. 1976. Maternal effects and selection response with an application to sheep data. Anim Prod 22:359-369.

Hardiman, J. W., W. E. Urban, and W. M. Collins. 1974. Analysis of inbreeding in selected and control lines of Japanese quail. Poultry Sci 53:1932-1932.

Harris, W. E., and T. Uller. 2009. Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. Phil Trans R Soc Lond B 364:1039-1048.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296.

Hassan, A., A, G., M. H. Hassanein, and A. H. Kamel. 1962. Biological studies in the Indian meal moth *Plodia interpunctella* (Hbn.). Bull Entomol Soc Egypt 46:233-256.

Head, M. L., J. Hunt, and R. Brooks. 2006. Genetic association between male attractiveness and female differential allocation. Biol Letters:341-344.

Heinrich, C. 1956. American moths of the subfamily Phycitinae. Smithsonian Institutions, Washington D.C.

Hernandez-Martinez, S., H. Lanz, M. H. Rodriguez, L. Gonzalez-Ceron, and V. Tsutsumi. 2002. Cellular-mediated reactions to foreign organisms inoculated into the hemocoel of *Anopheles albimanus* (Diptera : Culicidae). J Med Entomol 39:61-69.

Hetru, C., P. Bulet, S. Cociancich, J. L. Dimarcq, D. Hoffman, and J. A. Hoffman. 1994. Antibacterial peptides/polypeptides in the insect host defence: a comparision with vertebrate antibacterial peptides/polypeptides. Pp. 44-65 *in* J. A. Hoffman, C. A. J. Janeway, and S. Natori, eds. Phylogenetic Perspectives in Immunity: The Insect Host Defense. CRC Press, Boca Raton.

Hildemann, W. H. 1974. Phylogeny of immune responses in invertebrates. Life Sci 14:605-614.

Hildemann, W. H., I. S. Johnson, and P. L. Jokiel. 1979. Immunocompetence in the lowest Metazoan phylum - transplantation immunity in sponges. Science 204:420-422.

Hildemann, W. H., and A. L. Reddy. 1973. Phylogeny of immune responsiveness: marine invertebrates. Federation of American Societies for Experimental Biology 32:2188-2194.

Hoang, A. 2001. Immune response to parasitism reduces resistance of *Drosophila melanogaster* to desiccation and starvation. Evolution 55:2353-2358.

Hoelzel, A. R., J. Halley, S. J. O'Brien, C. Campagna, T. Arnborm, B. Le Boeuf, K. Ralls, and G. A. Dover. 1993. Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. J Hered 84:443-449.

Hoffmann, J. A., and C. Hetru. 1992. Insect defensins - inducible antibacterial peptides. Immunol Today 13:411-415.

Hoffmann, J. A., and J. M. Reichhart. 2002. *Drosophila* innate immunity: an evolutionary perspective. Nat Immunol 3:121-126.

Hoffmann, J. A., J. M. Reichhart, and C. Hetru. 1996. Innate immunity in higher insects. Curr Opin Immunol 8:8-13.

Holbrook, G. L., and C. Schal. 2004. Maternal investment affects offspring phenotypic plasticity in a viviparous cockroach. Proc Natl Acad Sci USA 101:5595-5597.

Hollingsworth, M. J., and J. Maynard Smith. 1955. The effects of inbreeding on rate of development and on fertility in *Drosophila subobscura*. J Genet 53:295-314.

Honek, A. 1993. Intraspecific Variation in Body Size and Fecundity in Insects - a General Relationship. Oikos 66:483-492.

Hopkins, T. L., and K. J. Kramer. 1992. Insect Cuticle Sclerotization. Annu Rev Entomol 37:273-302.

Horn, H. A., and M. S. Kerr. 1963. Hemolymph protein and copper concentrations of adult blue crabs (*Callinectes sapidus*, Rathbun). Biological Bulletin of the Marine Biological Laboratory at Woods Hole 125:499-507.

Huey, R. B., T. Wakefield, W. D. Crill, and G. W. Gilchrist. 1995. Within generation and between generation effects of temperature on early fecundity of *Drosophila melanogaster*. Heredity 74:216-223.

Hunt, J., and L. W. Simmons. 2007. Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. Evol Biol 54:936-941.

Huxham, I. M., and A. M. Lackie. 1988. Behavior in vitro of separated fractions of hemocytes of the Locust *Schistocerca gregaria*. Cell Tissue Res 251:677-684.

Iijima, R., S. Kurata, and S. Natori. 1993. Purification, characterization, and cDNA cloning of an antifungal protein from the hemolymph of *Sarcophaga peregrina* (Flesh Fly) Larvae. J Biol Chem 268:12055-12061.

Iketani, M., and I. Morishima. 1993. Induction of antibacterial protein synthesis by soluble peptidoglycan in isolated fat body from larvae of the Silkworm, *Bombyx mori*. Insect Biochem Molec 23:913-917.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biol Control 7:131-139.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1997a. Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of grasshoppers under field conditions. Environ Entomol 26:400-409.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1997b. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. Can Entomol 129:171-186.

Irving, P., L. Troxler, T. S. Heuer, M. Belvin, C. Kopczynski, J. M. Reichhart, J. A. Hoffmann, and C. Hetru. 2001. A genome-wide analysis of immune responses in Drosophila. Proc Natl Acad Sci USA 98:15119-15124.

Irving, P., J. M. Ubeda, D. Doucet, L. Troxler, M. Lagueux, D. Zachary, J. A. Hoffmann, C. Hetru, and M. Meister. 2005. New insights into *Drosophila* larval haemocyte functions through genome-wide analysis. Cell Microbiol 7:335-350.

Ishikawa, M., T. Kubo, and S. Natori. 1992. Purification and characterization of a Diptericin homolog from *Sarcophaga peregrina* (Flesh Fly). Biochem J 287:573-578.

Iwao, S. 1968. Some effects of grouping in Lepidopterous insects. Coll Int du Centre National de la Reserche Scientifique:185-210.

Jacobson, E. S., and H. S. Emery. 1991. Catecholamine uptake, melanization, and oxygen toxicity in *Cryptococcus neoformans*. J Bacteriol 173:401-403.

Jacobson, E. S., and S. B. Tinnell. 1993. Antioxidant function of fungal melanin. J Bacteriol 175:7102-7104.

Janeway, C. A., and R. Medzhitov. 1998. Introduction: The role of innate immunity in the adaptive immune response. Sem in Immunol 10:349-350.

Janeway, C. A., and R. Medzhitov. 1999. Innate immunity: lipoproteins take their Toll on the host. Curr Biol 9:R879-R882.

Janeway, C. A., and R. Medzhitov. 2002. Innate immune recognition. Annu Rev Immunol 20:197-216.

Janeway, C. A. J. 1994. The role of microbial pattern recognition in self: non-self discrimination in innate and adaptive immunity. Pp. 115-122 *in* J. A. Hoffmann, C. A. J. Janeway, and S. Natori, eds. Phylogenetic Perspectives in Immunity: The Insect Host Defense. CRC Press, Boca Raton.

Jaynes, J. 1989. Peptides to the Rescue. New Sci 124:42-44.

Jaynes, J. M., K. L. White, G. R. Julian, and F. M. Enright. 1989. Molecular biology and biochemistry of Cecropins and other novel lytic peptides. Abstr Pap Am Chem S 197:30-AGRO.

Jiravanichpaisal, P., K. Söderhäll, and I. Söderhäll. 2004. Effect of water temperature on the immune response and infectivity pattern of white spot syndrome virus (WSSV) in freshwater crayfish. Fish Shellfish Immun 17:265-275.

Johansson, A., P. Keyser, K. Sritunyalucksana, and K. Soderhall. 2000. Crustacean haemocytes and haematopoisis. Aquaculture 191:45-52.

Johansson, M. W., and K. Soderhall. 1989. Cellular-immunity in crustaceans and the Propo System. Parasitol Today 5:171-176.

Johnson, J. A., P. L. Wofford, and L. C. Whitehand. 1992. Effect of diet and temperature on development rates, survival, and reproduction of the Indian meal moth (Lepidoptera, Pyralidae). J Econ Entomol 85:561-566.

Johnson, K. S., and G. W. Felton. 2001. Plant phenolics as dietary antioxidants for herbivorous insects: a test with genetically modified tobacco. J Chem Ecol 27:2579-2597.

Johnson, S. J., L. D. Foil, A. M. Hammond, T. C. Sparks, and G. E. Church. 1985. Effects of environmental factors on phase variation in larval cotton leafworms, *Alabama argillacea* (Lepidoptera: Noctunidae). Ann Entomol Soc Am 78.

Jomori, T., and S. Natori. 1992. Function of the lipolysaccharide-binding protein of *Periplaneta americana* as an opsonin. Febs Lett 296:283-286.

Jung, Y. C., S. U. Kim, K. H. Son, H. H. Lee, and S. H. Bok. 1995. Isolation and Characterization of *Bacillus thuringiensis* Strain Bt-209 producing cuboidal delta-endotoxin crystals. J Microbiol Biotechn 5:138-142.

Kanost, M. R., M. S. Boguski, M. Freeman, J. I. Gordon, G. R. Wyatt, and M. A. Wells. 1988a. Primary structure of Apolipophorin-Iii from the migratory locust, *Locusta migratoria* - potential amphipathic structures and molecular evolution of an insect apolipoprotein. J Biol Chem 263:10568-10573.

Kanost, M. R., W. Dai, and P. E. Dunn. 1988b. Peptidoglycan fragments elicit antibacterial protein synthesis in larvae of *Manduca sexta*. Arch Insect Biochem 8:147-164.

Karban, R. 1988. Caterpillar basking behaviour and nonlethal parasitism by tachinid flies. J Insect Behav 11:714-723.

Kato, Y., Y. Motoi, K. Taniai, K. Kadonookuda, M. Hiramatsu, and M. Yamakawa. 1994. Clearance of lipopolysaccharide in hemolymph of the Silkworm *Bombyx mori* - its role in the termination of Cecropin messenger-RNA induction. Insect Biochem Molec 24:539-545.

Kawasaki, K., T. Kubo, and S. Natori. 1993. A novel role of *Periplaneta* lectin as an opsonin to recognize 2-Keto-3-Deoxy octonate residues of bacterial lipopolysaccharides. Comp Biochem Phys B 106:675-680.

Keppi, E., J. L. Dimarcq, J. Lambert, D. Zachary, and D. Hoffmann. 1986. Diptericin-a, a novel antibacterial peptide induced by immunization or injury in larvae of the dipteran insect *Phormia terranovae*. Immunobiology 173:229-230.

Kerridge, P. M. T. 1926. The buffering power of the blood of *Maia squinado*. J Physiol 62:65-73.

Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. Evolution 43:485-503.

Kittelson, P. M., and J. L. Maron. 2000. Outcrossing rate and inbreeding depression in the perennial yellow bush lupine, *Lupinus arboreus* (Fabaceae). Am J Bot 87:652-660.

Kleinholz, L. H. 1985. Biochemistry of crustacean hormones. Pp. 295-296 *in* D. E. Bliss, and L. H. Mantel, eds. The Biology of Crustacea. Academic Press, New York.

Kobayashi, A., M. Matsui, T. Kubo, and S. Natori. 1993. Purification and characterization of a 59-kilodalton protein that specifically binds to Nf-kappa-B-binding motifs of the defense protein genes of *Sarcophaga peregrina* (the Flesh Fly). Mol Cell Biol 13:4049-4056.

Kobayashi, M., S. Ingaki, and S. Kawase. 1981. Effect of high temperature on the development of nuclear polyhedrosis virus in the silkworm, *Bombyx mori*. J Invertebr Pathol 38:386-394.

Koelle, K., M. A. Pascual, and M. Yunus. 2005. Pathogen adaption to seasonal forcing and climate change. Proc R Soc B - Biological Sciences 272:971-977.

Kolm, N. 2001. Females produce larger eggs for large males in a paternal mouthbrooding fish. Proc R Soc Lond B Biological Sciences 268:2229-2234.

Kondo, R., Y. Satta, E. T. Matsuura, H. Ishiwa, N. Takahata, and S. I. Chigusa. 1990. Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics 126:657-663.

Kraaijeveld, A. R., J. Ferrari, and H. C. J. Godfray. 2002. Costs of resistance in insect-parasite and insect-parasitoid interactions. Parasitology 125:S71-S82.

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

Kraaijeveld, A. R., E. C. Limentani, and H. C. J. Godfray. 2001. Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Proc R Soc Lond, Series B - Biological Sciences 268:259-261.

Kubi, C., J. Van den Abbeele, R. De Deken, T. Marcotty, P. Dorny, and P. Van den Bossche. 2006. The effect of starvation on the susceptibility of teneral and non-teneral tsetse flies to trypanosome infection. Med Vet Entomol 20:388-392.

Kunimi, Y., and E. Yamada. 1990. Relationship of larval phase and susceptibility of the armyworm, *Pseudaletia separata* Walker (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus and a granulosis virus. Appl Entomol Zool 25:289-297.

Kuo, M., and M. Alexander. 1967. Inhibition of the lysis of fungi by melanins. J Bacteriol 94:624-629.

Kurtz, J. 2005. Specific memory within innate immune systems. Trends Immunol 26:186-192.

Kurtz, J., and K. Franz. 2003. Evidence for memory in invertebrate immunity. Nature 425:37-38.

Kurtz, K., A. Wiesner, P. Gotz, and K. Sauer. 2000. Gender differences and individual variation in the immune system of the scorpionfly *Panorpa vulgaris* (insecta: mecoptera). Dev Comp Immunol 24:1-12.

Kyriakides, T. R., J. L. Mckillip, and K. D. Spence. 1995. Biochemical-characterization, developmental expression, and induction of the immune protein Scolexin from *Manduca sexta*. Arch Insect Biochem 29:269-280.

Lackie, A. M. 1988. Haemocyte Behaviour. Advs Insect Physiol 21:85-178.

Ladendorff, N. E., and M. R. Kanost. 1990. Isolation and characterization of bacteria-induced protein-P4 from hemolymph of *Manduca sexta.* Arch Insect Biochem 15:33-41.

Lambert, J., E. Keppi, J. L. Dimarcq, C. Wicker, J. M. Reichhart, B. Dunbar, P. Lepage, A. Vandorsselaer, J. Hoffmann, J. Fothergill, and D. Hoffmann. 1989. Insect immunity - isolation from immune blood of the Dipteran *Phormia terranovae* of 2 insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. Proc Natl Acad Sci USA 86:262-266.

Lande, R., and K. Ritland. 1994. The relationship between inbreeding dperession and prior inbreeding among populations of four *Mimulus* taxa. Evolution 48:806-817.

Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434-437.

Lanot, R., D. Zachary, F. Holder, and M. Meister. 2001. Postembryonic hematopoiesis in *Drosophila*. Dev Biol 230:243-257.

Lavine, M. D., and M. R. Strand. 2002. Insect hemocytes and their role in immunity. Insect Biochem Molec 32:1295-1309.

LeCato, G. L. 1976. Yield, development and weight of *Cadra cautella* (Walker) and *Plodia interpunctella* (Hubner) on twenty-one diets derived from natural products. J Stored Prod Res 12:43-47.

Lee, D. K., B. S. Kim, D. H. Kim, S. Kim, J. H. Chung, D. M. Han, B. L. Lee, and Y. Lee. 1995. Expression of an insect antifungal protein of *Tenebrio molitor* in *Escherichia coli*. Mol Cells 5:429-435.

Lee, K. P., J. S. Cory, K. Wilson, D. Raubenheimer, and S. J. Simpson. 2006. Flexible diet choice offsets protein costs of pathogen resistence in a caterpillar. Proc R Soc Lond B-Biological Sciences 273:823-829.

Lee, K. P., D. Raubenheimer, S. T. Behmer, and S. J. Simpson. 2003. A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). J Insect Physiol 49:1161-1171.

Lee, K. P., S. J. Simpson, and K. Wilson. 2008. Dietary protein-quality influences melanization and immune function in an insect. Funct Ecol 22:1052-1061.

Lee, W. J., and P. T. Brey. 1995. Isolation and characterization of the lysozyme-encoding gene from the Silkworm *Bombyx mori*. Gene 161:199-203.

Lemaitre, B., E. Kromermetzger, L. Michaut, E. Nicolas, M. Meister, P. Georgel, J. M. Reichhart, and J. A. Hoffmann. 1995. A recessive mutation, Immune-Deficiency (Imd), defines 2 distinct control pathways in the *Drosophila* host defense. Proc Natl Acad Sci USA 92:9465-9469.

Lemaitre, B., J. M. Reichhart, and J. A. Hoffmann. 1997. *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. P Natl Acad Sci USA 94:14614-14619.

Leonard, C., N. A. Ratcliffe, and A. F. Rowley. 1985. The Role of prophenoloxidase activation in non-self recognition and phagocytosis by insect blood cells. J Insect Physiol 31:789-799.

Leopold, R. A. 1976. Role of male accessory glands in insect reproduction. Annu Rev Entomol 21:199-221.

Leulier, F., C. Parquet, S. Pili-Floury, J. H. Ryu, M. Caroff, W. J. Lee, D. Mengin-Lecreulx, and B. Lemaitre. 2003. The *Drosophila* immune system detects bacteria through specific peptidoglycan recognition. Nat Immunol 4:478-484.

Levashina, E. A., S. Ohresser, P. Bulet, J. M. Reichhart, C. Hetru, and J. A. Hoffmann. 1995. Metchnikowin, a novel immune-inducible proline-rich peptide from *Drosophila* with antibacterial and antifungal Properties. Eur J Biochem 233:694-700.

Lindstromdinnetz, I., S. C. Sun, and I. Faye. 1995. Structure and expression of Hemolin, an insect member of the immunoglobulin gene superfamily. Eur J Biochem 230:920-925.

Little, T. J., and A. R. Kraaijeveld. 2004. Ecological and evolutionary implications of immunological priming in invertebrates. Trends Ecol Evol 19:58-60.

Little, T. J., B. O'Connor, N. Colegrave, K. Watt, and A. F. Read. 2003. Maternal transfer of strain-specific immunity in an invertebrate. Curr Biol 13:489-492.

Loker, E. S., C. M. Adema, S. M. Zhang, and T. B. Kepler. 2004. Invertebrate immune systems - not homogeneous, not simple, not well understood. Immunol Rev 198:10-24.

Lombardi, J. 1996. Postzygotic maternal influences and the maternal-embryonic relationship of viviparous fishes. Am Zool 36:106-115.

Long, D. B. 1953. Effcts of population density on larvae of Lepidoptera. T Roy Ent Soc London 104:543-585.

Lynch, M., and J. B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland, MA.

Ma, C., H. Jiang, Y. Wang, and M. R. Kanost. 2000. Characterization of two pattern recognition molecules in the plasma of the tobacco hornworm, *Manduca sexta*. Faseb J 14:A1391-A1391.

Ma, C. C., and M. R. Kanost. 2000. A beta 1,3-glucan recognition protein from an insect, *Manduca sexta*, agglutinates microorganisms and activates the phenoloxidase cascade. J Biol Chem 275:7505-7514.

Ma, C. C., and M. R. Kanost. 2001. A beta-1,3-glucan-binding protein from *Manduca sexta*. Phylogenetic Perspectives on the Vertebrate Immune System 484:309-312.

Magiafoglou, A., and A. A. Hoffmann. 2003. Cross-generation effects due to cold exposure in *Drosophila serrata*. Funct Ecol 17:664-672.

Manwell, C., and C. M. A. Baker. 1963. Starch gel electrophoresis of sera from some marine arthropods: studies on the heterogeneity of haemocyanin and on a "ceruloplasmin-like" protein. Comp Biochem Physiol 8:193-208.

Marmaras, V. J., and N. Charalambidis. 1992. Certain hemocyte proteins of the Medfly, *Ceratitis capitata,* are responsible for nonself recognition and immobilization of *Escherichia coli* in vitro. Arch Insect Biochem 21:281-288.

Marmaras, V. J., N. D. Charalambidis, and M. Lambropoulou. 1994. Cellular defense mechanisms in C. capitata - recognition and entrapment of *Escherichia coli* by hemocytes. Arch Insect Biochem 26:1-14.

Marriott, C. G., and G. J. Holloway. 1998. Colour pattern plasticity in the hoverfly, *Episyrphus balteatus*: The critical immature stage and reaction norm on developmental temperature. J Insect Physiol 44:113-119.

Mason, H. S. 1955. Comparative biochemistry of the phenolase complex. Advances in Enzymology and Related Subjects of Biochemistry 16:105-184.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft, and S. J. Simpson. 2005. Nutrient specific foraging in invertebrate predators. Science 307:111-113.

Mayntz, D., and S. Toft. 2001. Nutrient composition of the prey's diet affects growth and survivorship of a generalist predator. Oecologia 127:207-213.

Mazet, I., J. Pendland, and D. Boucias. 1994. Comparative analysis of phagocytosis of fungal cells by insect hemocytes versus horse neutrophils. Dev Comp Immunol 18:455-466.

Mazuc, J., O. Chastel, and G. Sorci. 2003. No evidence for differential maternal allocation to offspring in the house sparrow (*Passer domesticus*). Behav Ecol 14:340-346.

Mbata, G. N., and F. N. C. Osuji. 1983. Some aspects of the biology of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae), a pest of stored groundnuts in Nigeria. J Stored Prod Res 19:141-151.

McClain, E., P. Magnuson, and S. J. Warner. 1988. Behavioural fever in a Namibian desert tenebrionid beetle, *Onymacris plana*. J Insect Physiol 34:279-284.

McGaughey, W. H. 1978. Effects of larval age on the susceptibility of Almond moths and Indian meal moths to *Bacillus thuringiensis*. J Econ Entomol 71:923-925.

Mcginley, M. A., D. H. Temme, and M. A. Geber. 1987. Parental Investment in offspring in variable environments - theoretical and empirical considerations. Am Nat 130:370-398.

McKean, K. A., and L. Nunney. 2005. Batemans principle and immunity: phenotypicaly plastic reproductive startegies predict changes in immunological sex differences. Evolution 59:1510-1517.

Medzhitov, R., P. PrestonHurlburt, and C. A. Janeway. 1997. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature 388:394-397.

Meister, M., and M. Lagueux. 2003. *Drosophila* blood cells. Cell Microbiol 5:573-580.

Menti, H., D. J. Wright, and R. N. Perry. 2000. Infectivity of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* in relation to temperature, age and lipid content. Nematology 2.

Metcalfe, N., B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254-260.

Metz, B., O. R. Davidson, P. R. Bosch, R. Dave, L. A. Meyer, and (Eds). 2007. IPCC, 2007. Pp. 851. Climate Change 2007: Mitigation of Climate Change. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York.

Mitsui, J., and Y. Kunimi. 1988. Effect of larval phase on susceptibility of the Armyworm, *Pseudaletia separata* Walker (Lepidoptera, Noctuidae) to an entomogeneous deuteromycete, *Nomuraea rileyi*. Jpn J Appl Entomol Z 32:129-134.

Mody, K., S. B. Unsicker, and K. E. Linsenmair. 2007. Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. Ecology 88:1012-1020.

Moret, Y., and P. Schmid-Hempel. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1161-1168.

Moret, Y., and P. Schmid-Hempel. 2001. Entomology - immune defence in bumble-bee offspring. Nature 414:506-506.

Moret, Y., and M. T. Siva-Jothy. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. Proc R Soc Lond B Biological Sciences 270:2475-2480.

Morishima, I., T. Horiba, M. Iketani, E. Nishioka, and Y. Yamano. 1995. Parallel induction of cecropin and lysozyme in larvae of the silkworm, *Bombyx mori*. Dev Comp Immunol 19:357-363.

Morishima, I., K. Yamada, and T. Ueno. 1992. Bacterial peptidoglycan as elicitor of antibacterial protein synthesis in larvae of the Silkworm, *Bombyx mori.* Insect Biochem Molec 22:363-367.

Mousseau, T. A., and H. Dingle. 1991. Maternal effects in insect life histories. Annu Rev Entomol 36:511-534.

Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. Trends Ecol Evol 13:403-407.

Mulnix, A. B., and P. E. Dunn. 1994. Structure and Induction of a lysozyme gene from the Tobacco Hornworm, *Manduca sexta*. Insect Biochem Molec 24:271-281.

Muta, T., and S. Iwanaga. 1996. The role of hemolymph coagulation in innate immunity. Curr Opin Immunol 8:41-47.

Mydlarz, L. D., L. E. Jones, and C. D. Harvell. 2006. Innate immunity, environmental drivers, and disease ecology of marine and freshwater invertebrates. Annu Rev Ecol Evol Systemat 37:251-288.

Na, J. H., and M. I. Ryoo. 2000. The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. J Stored Prod Res 36:125-129.

Nappi, A. J., E. Vass, F. Frey, and Y. Carton. 1995a. Superoxide anion generation in Drosophila during melanotic encapsulation of parasites. Eur J Cell Biol 68:450-456.

Nappi, A. J., E. Vass, F. Frey, and Y. Carton. 1995b. Superoxide anion generation in *Drosophila* during melanotic encapsulation of parasites. Eur J Cell Biol 68:450-456.

O'Brien, S. J., M. Roelke, L. Marker, A. Newman, C. A. Wikler, D. Meltzer, L. Colly, J. F. Everman, M. Bush, and D. E. Widt. 1985. Genetic basis for species vulnerability in the cheetah. Science 227:1428-1434.

Ochiai, M., and M. Ashida. 1999. A pattern recognition protein for peptidoglycan - Cloning the cDNA and the gene of the silkworm, *Bombyx mori*. J Biol Chem 274:11854-11858.

Ochiai, M., T. Niki, and M. Ashida. 1992. Immunocytochemical localization of beta-1,3-glucan recognition protein in the Silkworm, *Bombyx mori.* Cell Tissue Res 268:431-437.

Ojala, K., R. Julkunen-Tiito, L. Lindstrom, and J. Mappes. 2005. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis.* Evol Ecol Res 7:1153-1170.

Oksanen, T. A., R. V. Alatalo, T. J. Horne, E. Koskela, J. Mappes, and T. Mappes. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. P Roy Soc Lond B Bio 266:1495-1499.

Olsen, L. E., and M. A. Hoy. 2002. Heat curing *Metaseilus occidentalis* (Nesbit) (Acari, Phytoseiidae) of a fitness-reducing microsporidium. J Invertebr Pathol 79:173-178.

Ourth, D. D., and H. E. Renis. 1993a. Antiviral melanisation reaction of *Heliothis virescens* haemolymph against DNA and RNA viruses in vitro. Comp Biochem Physiol 105B:719-723.

Ourth, D. D., and H. E. Renis. 1993b. Antiviral melanization reaction of Heliothis virescens hemolymph against DNA and RNA viruses in vitro. Comp Biochem Phys B 105:719-723.

Pal, C., and I. Miklos. 1999. Epigenetic inheritance, genetic assimilation and speciation. J Theor Biol 200:19-37.

Pawelek, J. M., and A. B. Lerner. 1978. 5,6-Dihydroxyindole is a melanin precursor showing potent cytotoxicity. Nature 276:627-628.

Pech, L. L., and M. R. Strand. 1996. Granular cells are required for encapsulation of foreign targets by insect haemocytes. J Cell Sci 109:2053-2060.

Pech, L. L., and M. R. Strand. 2000. Plasmatocytes from the moth Pseudoplusia includens induce apoptosis of granular cells. J Insect Physiol 46:1565-1573.

Pendland, J. C., and D. G. Boucias. 1996. Phagocytosis of lectin-opsonized fungal cells and endocytosis of the ligand by insect *Spodoptera exigua* granular hemocytes: An ultrastructural and immunocytochemical study. Cell Tissue Res 285:57-67.

Pener, M. P., and Y. Yerushalmi. 1998. The physiology of locust phase polymorphism: an update. J Insect Physiol 44:365-377.

Perez-Mendoza, J., and M. Aguilera-Pen. 2004. Development, reproduction,and control of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), in stored seed garlic in Mexico. J Stored Prod Res 40:409-421.

Petrie, M., and A. Williams. 1993. Peahens Lay More Eggs for Peacocks with Larger Trains. Proc R Soc Lond B Biological Sciences 251:127-131.

Phipps, D. J., J. S. Chadwick, and W. P. Aston. 1994. Gallysin-1, an antibacterial protein isolated from hemolymph of *Galleria mellonella*. Dev Comp Immunol 18:13-23.

Pianka, E. R. 1972. r and K selection or b and d selection. The American Naturalist 106:581-588.

Podoler, H. 1974. Analysis of life-tables for a host and parasite (Plodia-Nemeritis) ecosystem. J Anim Ecol 43:653-670.

Pounds, J. A. 2001. Climate and amphibian declines. Nature 410:639-640.

Povey, S., S. C. Cotter, S. J. Simpson, K. P. Lee, and K. Wilson. 2009. Can the protein costs of bacterial resistence be offest by altered feeding behaviour? J Anim Ecol 78:437-446.

Rantala, M. J., and D. A. Roff. 2006. Analysis of the importance of genotypic variation, metabolic rate, morphology, sex and development time on immune function in the cricket, *Gryllus firmus*. J Evolution Biol 19:834-843.

Rantala, M. J., and D. A. Roff. 2007. Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. Heredity 98:329-336.

Ratcliffe, N. A., and S. J. Gagen. 1976. Cellular defense reactions of insect hemocytes in vivo - nodule formation and development in *Galleria mellonella* and *Pieris brassicae* larvae. J Invertebr Pathol 28:373-382.

Ratcliffe, N. A., and S. J. Gagen. 1977. Studies on in vivo cellular reactions of insects - ultrastructural analysis of nodule formation in *Galleria mellonella*. Tissue Cell 9:73-85.

Ratcliffe, N. A., C. Leonard, and A. F. Rowley. 1984. Prophenoloxidase activation - nonself recognition and cell cooperation in insect immunity. Science 226:557-559.

Ratcliffe, N. A., and A. F. Rowley. 1979. Comparative synopsis of the structure and function of the blood cells of insects and other invertebrates. Dev Comp Immunol 3:189-221.

Reaney, L. T., and R. J. Knell. 2010. Immune activation but not male quality affects female current reproductive investment in a dung beetle. Behav Ecol 21:1367-1372.

Reeson, A. F., K. Wilson, J. S. Cory, P. Hankard, J. M. Weeks, D. Goulson, and R. S. Hails. 2000. Effects of phenotypic plasticity on pathogen transmission in the field in a Lepidoptera-NPV system. Oecologia 124:373-380.

Reeson, A. F., K. Wilson, A. Gunn, R. S. Hails, and D. Goulson. 1998a. Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. Proc R Soc Lond B Biological Sciences 265:1787-1791.

Reeson, A. F., K. Wilson, A. Gunn, R. S. Hails, and D. Goulson. 1998b. Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. Proc R Soc Lond, Series B-Biological Sciences 265:1787-1791.

Reichhart, J. M., M. Essrich, J. L. Dimarcq, D. Hoffmann, J. A. Hoffmann, and M. Lagueux. 1989. Insect immunity - isolation of Cdna Clones corresponding to Diptericin, an inducible antibacterial peptide from *Phormia terranovae* (Diptera) - transcriptional profiles during immunization. Eur J Biochem 182:423-427.

Reid, J. M., P. Arcese, and L. F. Keller. 2003. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and inter-generational effects. Proc R Soc Lond B Biological Sciences 270:2151-2157.

Reinhold, K. 1998. Paternal investment in *Poecilimon veluchianus* bushcrickets: beneficial effects of nuptial feeding on offspring viability. Behav Ecol Sociobiol 45:293-299.

Reinhold, K., and K. G. Heller. 1993. The ultimate function of nuptial feeding in the Bush-Cricket *Poecilimon veluchianus* (Orthoptera, Tettigoniidae, Phaneropterinae). Behav Ecol Sociobiol 32:55-60.

Reyes, A. V. 1969. Biology and host range of *Plodia interpunctella* Hubner (Pyralidae: Lepidoptera). Philippine Entomologist 1:301-311.

Rheins, L. A., R. D. Karp, and A. Butz. 1980. Induction of Specific Humoral Immunity to Soluble-Proteins in the American Cockroach (*Periplaneta americana*). 1. Nature of the Primary Response. Dev Comp Immunol 4:447-458.

Ribeiro, C., and M. Brehelin. 2006. Insect haemocytes: What type of cell is that? J Insect Physiol 52:417-429.

Ribeiro, C., N. Simoes, and M. Brehelin. 1996. Insect immunity: The haemocytes of the armyworm *Mythimna unipuncta* (Lepidoptera: Noctuidae) and their role in defence reactions. In vivo and in vitro studies. J Insect Physiol 42:815-822.

Rideout, R. M., E. A. Trippel, and M. Litvak, K. 2004. Paternal effects on haddock early life history traits. J Fish Biol 64:695-701.

Rigby, M. C., and J. Jokela. 2000. Predator avoidance and immune defence: costs and trade-offs in snails. Proc R Soc B-Biol Sci 267:171-176.

Riska, B., J. J. Rutledge, and W. R. Atchley. 1985. Covariance between direct and maternal genetic-effects in mice, with a model of persistent environmental-influences. Genet Res 45:287-297.

Roff, D. A., S. Mostowy, and D. J. Fairbairn. 2002. The evolution of trade-offs: testing predictions on response to selection and environmental variation. Evolution 56:84-95.

Rohloff, L. H., A. Wiesner, and P. Gotz. 1994. A fluorescence assay demonstrating stimulation of phagocytosis by hemolymph molecules of *Galleria mellonella*. J Insect Physiol 40:1045-1049.

Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate Ecological Immunology. Science 301:472-475.

Roosenburg, W. M., and K. C. Kelley. 1996. The effect of egg size and ioncubation temperature on growth in the Turtle, *Malaclemys terrapin*. J Herpetol 30:198-204.

Rossiter, M. C. 1991. Environmentally-based maternal effects - a hidden force in insect population dynamics. Oecologia 87:288-294.

Rossiter, M. C. 1996. Incidence and consequences of inherited environmental effects. Annu Rev Ecol Syst 27:451-476.

Roth, O., G. Joop, H. Eggert, J. Hilbert, J. Daniel, P. Schmid-Hempel, and J. Kurtz. 2010a. Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. J Anim Ecol 79:403-413.

Roth, O., G. Joop, H. Eggert, J. Hilbert, J. Daniel, P. Schmid-Hempel, and J. Kurtz. 2010b. Transgenerational immune priming as cryptic parental care. J Anim Ecol 79:722-722.

Roth, O., B. M. Sadd, P. Schmid-Hempel, and J. Kurtz. 2009. Strain-specific priming of resistance in the red flour beetle, Tribolium castaneum. Pro R Soc B-Biol Sci 276:145-151.

Russell, V., and P. E. Dunn. 1996. Antibacterial proteins in the midgut of *Manduca sexta* during metamorphosis. J Insect Physiol 42:65-71.

Russell, V. W., and P. E. Dunn. 1991. Lysozyme in the midgut of *Manduca sexta* during Metamorphosis. Arch Insect Biochem 17:67-80.

Russo, J., S. Dupas, F. Frey, Y. Carton, and M. Brehelin. 1995. Insect immunity: early events in the encapsulation process of parasitoid (*Leptinopilina boulardi*) eggs in resistant and susceptible strains of *Drosphila*. Parasitology 112:135-142.

Rutschmann, S., A. C. Jung, R. Zhou, N. Silverman, J. A. Hoffmann, and D. Ferrandon. 2000. Role of *Drosophila* IKK gamma in a Toll-independent antibacterial immune response. Nat Immunol 1:342-347.

Rutschmann, S., A. Kilinc, and D. Ferrandon. 2002. Cutting edge: The Toll pathway is required for resistance to Gram-positive bacterial infections in *Drosophila.* J Immunol 168:1542-1546.

Rutstein, A. N., L. Gilbert, P. J. B. Slater, and J. A. Graves. 2004. Mate attractiveness and primary resource allocation in the zebra finch. Anim Behav 68:1087-1094.

Saccheri, I. J., P. M. Brakefield, and R. A. Nichols. 1996. Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). Evolution 50:2000-2013.

Sackton, T. B., B. P. Lazzaro, and A. G. Clark. 2010. Genotype and gene expression associations with immune function in *Drosophila*. PLoS Genet 6:e1000797.

Sadd, B. M., Y. Kleinlogel, R. Schmid-Hempel, and P. Schmid-Hempel. 2005. Trans-generational immune priming in a social insect. Biol Letters 1:386-388.

Sadd, B. M., and M. T. Siva-Jothy. 2006. Self-harm caused by an insect's innate immunity. Proc R Soc B-Biol Sci 273:2571-2574.

Samakovlis, C., B. Asling, H. G. Boman, E. Gateff, and D. Hultmark. 1992. Invitro induction of Cecropin Genes - an immune-response in a *Drosophila* Blood-Cell line. Biochem Bioph Res Co 188:1169-1175.

Schmid-Hempel, P., K. Puhr, N. Kruger, C. Reber, and R. Schmid-Hempel. 1999. Dynamic and genetic consequences of variation in horizontal transmission for a microparasitic infection. Evolution 53:426-434.

Schmidt, H. U. 1982. The depth of egg deposition of the Indian meal moth, *Plodia interpunctella* Hbn. in rye and maize. Anz Schadlingskd Pfl 55:1-4.

Schmit, A. R., and N. A. Ratcliffe. 1977. Encapsulation of foreign tissue implants in *Galleria mellonella* Larvae. J Insect Physiol 23:175-184.

Schnare, M., G. M. Barton, A. C. Holt, K. Takeda, S. Akira, and R. Medzhitov. 2001. Toll-like receptors control activation of adaptive immune responses. Nat Immunol 2:947-950.

Schoffeniels, E. 1976. Adaptations with respect to salinity. Biochemical Society Symposium 41:179-204.

Schwarzenbach, G. A., and P. I. Ward. 2006. Responses to selection on phenoloxidase activity in yellow dung flies. Evolution 60:1612-1621.

Sedlacek, J. D., P. A. Weston, and J. Barney. 1996. Lepidoptera and Psocoptera. Pp. 41-70 *in* B. Subramanyam, and D. W. Hagstrum, eds. Integrated Management of Insects in Stored Products. Marcel Dekker Inc., New York.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317-321.

Sigsgaard, L. 2000. The temperature-dependent duration of development and parasitism of three cereal aphid parasitoids, *Aphidius ervi*, *A. rhopalosiphi* and *Praon volucre*. Entomol Exp Appl 95:173-184.

Silhacek, D. L., and G. L. Miller. 1972. Growth and development of the Indian meal moth, *Plodia interpunctella* (Leptidoptera: Pyralidae), under laboratory mass-rearing conditions. Annual Meeting of the Entomological Society of America 65:1084-1087.

Simmons, L. W. 1990. Nuptial feeding in tettigoniids male costs and the rates of fecundity increase Behav Ecol Sociobiol 27:43-47.

Simmons, M. J., and J. F. Crow. 1977. Mutations affecting fitness in *Drosophila* populations. Annu Rev Genet 11:49-78.

Sinervo, B., and D. F. DeNardo. 1996. Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. Evolution 50:1299-1313.

Sinervo, B., and L. R. Mcedward. 1988. Developmental consequences of an evolutionary change in egg size - an experimental test. Evolution 42:885-899.

Siva-Jothy, M. T., and J. J. W. Thompson. 2002. Short-term nutrient deprivation affects immune function. Physiol Entomol 27:206-212.

Slatis, H. 1959. An analysis of inbreeding in the european bison. Genetics 45.

Soderhall, K. 1982. Prophenoloxidase activating system and melanization - a recognition mechanism of arthropods - a review. Dev Comp Immunol 6:601-611.

Soderhall, K., and R. Ajaxon. 1982. Effect of quinones and melanin on mycelial gowth of *Aphanomyces* spp. and extracellular erotease of *Aphanomyces astaci* a parasite on crayfish. J Invertebr Pathol 39:105-109.

Soderhall, K., and L. Hall. 1984. Lipopolysaccharide-induced activation of prophenoloxidase activating system in Crayfish hemocyte lysate. Biochim Biophys Acta 797:99-104.

Soderhall, K., and V. J. Smith. 1983. Separation of the hemocyte populations of *Carcinus maenas* and other marine decapods, and prophenoloxidase distribution. Dev Comp Immunol 7:229-239.

Soderhall, K., V. J. Smith, and M. W. Johansson. 1986. Exocytosis and uptake of bacteria by isolated hemocyte populations of 2 crustaceans - evidence for cellular cooperation in the defense reactions of arthropods. Cell Tissue Res 245:43-49.

Sperlich, D., H. Feuerbachmravlag, P. Lange, A. Michaelidis, and A. Pentzosdaponte. 1977. Genetic load and viability distribution in central and marginal populations of *Drosophila subobscura*. Genetics 86:835-848.

Srygley, R. B., P. D. Lorch, S. J. Simpson, and G. A. Sword. 2009. Immediate protein dietary effects on movement and the generalised immunocompetence of migrating Mormon crickets *Anabrus simplex* (Orthoptera: Tettigoniidae). Ecol Entomol 34:663-668.

St Leger, R. J., L. Joshi, and D. Roberts. 1998. Ambient pH is a major determinant in the expression of cuticle-degrading enzymes and hydrophobin by *Metarhizium anisopliae*. Appl Environ Microb 64:709-713.

Stamp, N. E., and M. D. Bowers. 1990. Variation in food quality and temperature constrain foraging of gregarious caterpillars. Ecology 71:1031-1039.

Steinhaus, E. A. 1958. Crowding as a possible stress factor in insect disease. Ecology 39:503-514.

Stevens, L., G. Yan, and L. A. Pray. 1997. Consequences of inbreeding on invertebrate host susceptibility to parasitic infection. Evolution 51:2032-2039.

Strand, M. R. 2008. The insect cellular immune response. Insect Sci 15:1-14.

Strand, M. R., and L. L. Pech. 1995. Immunological basis for compatibility in parasitoid host relationships. Annu Rev Entomol 40:31-56.

Strathmann, R. R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine-invertebrates. Annu Rev Ecol Syst 16:339-361.

Sugumaran, H. 2002a. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. Pigm Cell Res 15:2-9.

Sugumaran, M. 1996. Roles of the insect cuticle in immunity. Pp. 355-374 *in* K. Soderhall, S. Iwanaga, and G. Vastha, eds. New Directions in Invertebrate Immunology. SOS Publications, Fair Haven, NJ.

Sugumaran, M. 1998. Unified mechanism for sclerotization of insect cuticle. Advs Insect Physiol 27:229-334.

Sugumaran, M. 2002b. Mechanistic studies on insect cuticular hardening and extrapolation to green chemistry application. Abstr Pap Am Chem S 224:U37-U37.

Sugumaran, M., S. Tan, and H. L. Sun. 1996. Tyrosinase-catalyzed oxidation of 3,4-dihydroxyphenylglycine. Arch Biochem Biophys 329:175-180.

Sun, S. C., and I. Faye. 1995. Transcription of immune genes in the Giant Silkmoth, *Hyalophora cecropia* is augmented by H2o2 and diminished by thiol reagents. Eur J Biochem 231:93-98.

Swindell, W. R., and J. L. Bouzat. 2006a. Ancestral inbreeding reduces the magnitude of inbreeding depression in *Drosphila melanogaster.* Evolution 60:762-767.

Swindell, W. R., and J. L. Bouzat. 2006b. Reduced inbreeding depression due to historical inbreeding in Drosophila melanogaster: evidence for purging. J Evolution Biol 19:1257-1264.

Szymas, B., and A. Jedruszuk. 2002. The influence of different diets on haemocytes of adult worker honey bees, *Apis mellifera*. Apidologie 34:97-102.

Takeda, K., and S. Akira. 2003. Toll receptors and pathogen resistance. Cell Microbiol 5:143-153.

Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. Annu Rev Immunol 21:335-376.

Takeda, K., O. Takeuchi, and S. Akira. 2002. Recognition of lipopeptides by Toll-like receptors. J Endotoxin Res 8:459-463.

Telfer, W. H. 2009. Egg formation in Lepidoptera. J Insect Sci 9:-.
Templeton, A. R., and B. Read. 1984. Factors eliminating inbreeding depression in a captive herd of Spekes Gazelle (*Gazella spekei*). Zoo Biol 3:177-199.
Tojo, S., F. Naganuma, K. Arakawa, and S. Yokoo. 2000. Involvement of both granular cells and plasmatocytes in phagocytic reactions in the greater wax moth, *Galleria mellonella*. J Insect Physiol 46:1129-1135.
Tollrian, R. 1995. Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. Ecology 76:1691-1705.
Torres-Vila, L. M., and M. D. Jennions. 2004. Male mating history and female fecundity int he Lepidoptera: do male virgins make better partners? Behav Ecol Sociobiol 57:318-326.
Torres-Vila, L. M., M. C. Rodriguez-Molina, and M. D. Jennions. 2004. Polyandry and fecundity in the Lepidotera: can methological and conceptual approaches bias outcomes? Behav Ecol Sociobiol 55:315-324.
Trenczek, T., and H. Bennich. 1987. Insect immunity - antibacterial proteins in insect hemocytes. Scand J Immunol 26:332-332.
Trenczek, T., and I. Faye. 1988. Synthesis of immune proteins in primary cultures of fat body from *Hyalophora cecropia*. Insect Biochem 18:299-312.
Truscott, R., and K. N. White. 1990. The influence of metal and temperature stress on the immune system of crabs. Funct Ecol 4:455-461.
Tzanakakis, M. E. 1959. An ecological study of the Indian-meal moth *Plodia interpunctella* (Hübner) with emphasis on diapause. Hilgardia 29:205-246.
Uglow, R. F. 1969a. Haemolymph protein concentrations in portunid crabs. 1. Studies on adult *Carcinus maenus*. Comp Biochem Physiol 30:1083-1090.
Uglow, R. F. 1969b. Haemolymph protein concentrations in portunid crabs. 2. The effects of imposed fasting of *Carcinus maenus*. Comp Biochem Physiol 31:959-967.
Uglow, R. F. 1969c. Haemolymph protein concentrations in portunid crabs. 3. The effect of *Sacculina*. Comp Biochem Physiol 31:969-973.
Vahed, K. 1998. The function of nuptial feeding in insects: review of empirical studies. Biol Rev 73:43-78.
Vahed, K., and F. S. Gilbert. 1997. No effect of nuptial gift consumption on female reproductive output in the bushcricket *Leptophyes laticauda* Friv. Ecol Entomol 22:479-482.
Vainio, L., H. Hakkarainen, M. J. Rantala, and J. Sorvari. 2004. Individual variation in immune function in *Forica exsecta*: effect of nest, body size and sex. Evol Ecol 18:75-84.
Verhulst, P. F. 1838. Notice sur la loi que la population suit dans son accroissement. Corresp Math Phys 10:113-121.
Vierstraete, E., P. Verleyen, G. Baggerman, W. D'Hertog, G. Van den Bergh, L. Arckens, A. De Loof, and L. Schoofs. 2004a. A proteomic approach for the analysis of instantly released wound and immune proteins in *Drosophila melanogaster* hemolymph. P Natl Acad Sci USA 101:470-475.
Vierstraete, E., P. Verleyen, F. Sas, G. Van den Bergh, A. De Loof, L. Arckens, and L. Schoofs. 2004b. The instantly released *Drosophila* immune proteome is infection-specific. Biochem Bioph Res Co 317:1052-1060.

von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist, and H. Witzell. 1999. Good genes, oxidative stress, and condition dependent signals. Proceedings of the Royal Society of London B-Biological Sciences 266:1-12.

Wade, M. J., and R. W. Beeman. 1994. The population-dynamics of maternal-effect selfish genes. Genetics 138:1309-1314.

Wago, H. 1991. Phagocytic recognition in *Bombyx mori*. Pp. 215-235 *in* A. P. Gupta, ed. Immunology of insects and other arthropods. CRC Press, Boca Raton.

Wang, Y. L., P. Aisen, and A. Casadevall. 1995. *Cryptococcus neoformans* melanin and virulence - Mechanism of Action. Infect Immun 63:3131-3136.

Wang, Y. L., and A. Casadevall. 1994a. Decreased Susceptibility of Melanized Cryptococcus-Neoformans to Uv-Light. Appl Environ Microb 60:3864-3866.

Wang, Y. L., and A. Casadevall. 1994b. Susceptibility of melanized and nonmelanized *Cryptococcus neoformans* to nitrogen-derived and oxygen-derived oxidants. Infect Immun 62:3004-3007.

Washburn, J. O., B. A. Kirkpatrick, and L. E. Volkman. 1996. Insect protection against viruses. Nature 383:767-767.

Watson, D. W., B. A. Mullens, and J. J. Petersen. 1993. Behavioural fever response of *Musca domestica* (Diptera: Muscidae) to infection by *Enteromorpha muscae* (Zygomycetes: Entomorphthorales). J Invertebr Pathol 61:10-16.

Watson, M. J. O., and A. A. Hoffmann. 1996. Acclimation, cross-generation effects, and th response to selection for increased cold resistance in *Drosophila*. Evolution 50:1182-1192.

Webster, J. P., and M. E. J. Woolhouse. 1999. Cost of resistance: relationship between reduced fertility and increased resistance in a snail-schistosome host-parasite system. P Roy Soc B-Biol Sci 266:391-396.

Wedell, N. 1996. Mate quality affects reproductive effort in a paternally investing species. Am Nat 148:1075-1088.

Wedell, N., and A. Arak. 1989. The Wartbiter Spermatophore and its effect on female reproductive output (Orthoptera, Tettigoniidae, *Decticus verrucivorus*). Behav Ecol Sociobiol 24:117-125.

Weigensberg, I., Y. Carriere, and D. A. Roff. 1998. Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. J Evolution Biol 11:135-146.

Westneat, D. F., and T. R. Birkhead. 1998. Alternative hypotheses linking the immune system and mate choice for good genes. P Roy Soc Lond B Bio 265:1065-1073.

Whitlock, M. C. 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles and sexual selection. Evolution 54:1855-1861.

Wicker, C., J. M. Reichhart, D. Hoffmann, D. Hultmark, C. Samakovlis, and J. A. Hoffmann. 1990. Insect immunity - characterization of a *Drosophila* Cdna-encoding a novel member of the diptericin family of immune peptides. J Biol Chem 265:22493-22498.

Wiegand, C., D. Levin, J. P. Gillespie, E. Willott, M. R. Kanost, and T. Trenczek. 2000. Monoclonal antibody MS13 identifies a plasmatocyte membrane protein and inhibits encapsulation and spreading reactions of *Manduca sexta* hemocytes. Arch Insect Biochem 45:95-108.

Will, M. W., and S. K. Sakaluk. 1994. Courtship feeding in decorated crickets: is the spermatophylax a sham? Anim Behav 48:1309-1315.

Willham, R. L. 1972. The role of maternal effects in animal breeding: III. Biometrical aspects of maternal effects in animals. Am Soc Anim Sci 35:1288-1293.

Williams, G. C. 1964. The life-history of the Indian meal-moth, *Plodia interpunctella* (Hubner)(Lep. Phycitidae) in a warehouse in Britain and on different foods. Ann Appl Biol 53:459-475.

Willis, J. H. 1993. Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. Evolution 47:864-876.

Wilson, K. 2000. How the locust got its stripes: the evolution of density-dependent aposematism. Trends Ecol Evol 15:88-90.

Wilson, K., S. C. Cotter, A. F. Reeson, and J. K. Pell. 2001. Melanism and disease resistance in insects. Ecol Lett 4:637-649.

Wilson, K., R. Knell, M. Boots, and J. Koch-Osborne. 2003a. Group living and investment in immune defence: an interspecific analysis. J Anim Ecol 72:133-143.

Wilson, K., R. J. Knell, M. Boots, and J. Koch-Osborne. 2003b. Group living and investment in immune defence: an interspecific analysis. J Anim Ecol 72:133-143.

Wilson, K., and A. F. Reeson. 1998. Density-dependent prophylaxis: Evidence from Lepidoptera-baculovirus interactions Ecol Entomol 23:100-101.

Wilson, K., M. B. Thomas, S. Blanford, M. Doggett, S. J. Simpson, and S. L. Moore. 2002. Coping with Crowds: density-dependent disease resistance in desert locusts. Proc Natl Acad Sci USA 99:5471-5475.

Witteveldt, J., C. C. Cifuentes, J. M. Vlak, and M. C. W. van Hulten. 2004a. Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. J Virol 78:2057-2061.

Witteveldt, J., J. M. Vlak, and M. C. W. van Hulten. 2004b. Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. Fish Shellfish Immun 16:571-579.

Wolfner, M. F. 1997. Tokens of love: Functions and regulation of Drosophila male accessory gland products. Insect Biochem Molec 27:179-192.

Wolfner, M. F., H. A. Harada, M. J. Bertram, T. J. Stelick, K. W. Kraus, J. M. Kalb, Y. O. Lung, D. M. Neubaum, M. Park, and U. Tram. 1997. New genes for male accessory gland proteins in Drosophila melanogaster. Insect Biochem Molec 27:825-834.

Xu, J. H., M. Nishijima, Y. Kono, K. Taniai, Y. Kato, K. Kadonookuda, M. Yamamoto, M. Shimabukuro, S. Chowdhury, S. K. Choi, and M. Yamakawa. 1995. Identification of a Hemocyte Membrane-Protein of the Silkworm, Bombyx-Mori, Which Specifically Binds to Bacterial Lipopolysaccharide. Insect Biochem Molec 25:921-928.

Yokoo, S., P. Gotz, and S. Tojo. 1995. Phagocytic Activities of Hemocytes Separated by 2 Simple Methods from Larvae of 2 Lepidopteran Species, Agrotis-Segetum and Galleria-Mellonella. Appl Entomol Zool 30:343-350.

Yoshida, H., and M. Ashida. 1986. Peptidoglycan-Receptor in Prophenoloxidase Activating System in Insect. Zool Sci 3:1022-1022.

Yoshida, H., K. Kinoshita, and M. Ashida. 1996. Purification of a peptidoglycan recognition protein from hemolymph of the silkworm, Bombyx mori. J Biol Chem 271:13854-13860.

Zhang, R., H. Y. Cho, H. S. Kim, Y. G. Ma, T. Osaki, S. Kawabata, K. Soderhall, and B. L. Lee. 2003. Characterization and properties of a 1,3-beta-D-glucan pattern recognition protein of Tenebrio molitor larvae that is specifically degraded by serine protease during prophenoloxidase activation. J Biol Chem 278:42072-42079.

Zhang, S., C. M. Adema, T. B. Kepler, and E. S. Loker. 2004. Diversification of Ig Superfamily Genes in an Invertebrate Science 305:251-254.

Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. Am Nat 160:S9-S22.

Zuur, A. F. 2009. Mixed Effects Models and Extensions in Ecology with R (Statistics for Biology and Health). Springer, UK.