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Density-Dependent Prophylaxis in Insects

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Abstract

Parasites and pathogens are a ubiquitous threat facing all organisms. Life-history theory predicts that if investment in parasite resistance mechanisms is costly (as suggested by numerous studies), then organisms should tailor their investment in them to match their perceived risk of infection. Because most parasites are transmitted in a positively density-dependent manner, the threat from parasites tends to increase as population density increases. As a result, it is predicted that organisms should use population density as a cue to the risk of becoming infected and should increase investment in disease resistance mechanisms as the degree of crowding increases—this is known as *density-dependent prophylaxis* (DDP). This phenomenon has been experimentally tested in a number of insect species, and in most cases support for the DDP hypothesis has been forthcoming. DDP is likely to be particularly prevalent in species exhibiting density-dependent phase polyphenism (i.e. the phenotype adopted by the insect is plastic and dependent on the population density it experiences during its early development). We discuss the hormonal and genetic mechanisms underlying phase polyphenism and DDP, and speculate on the circumstances leading to their evolution. We end by discussing how future research into DDP might develop.

Introduction

All animals and plants share their biotic environment with parasites and pathogens (generically, 'parasites'). As a consequence, parasites represent

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an important selective force on their hosts. Indeed, they have been implicated in the evolution of host secondary sexual traits (e.g. Hamilton and Zuk 1982); the manipulation of host behavior (e.g. Moore 1984); the maintenance of host genetic diversity (e.g. van Valen 1973; Hamilton 1980) and the evolution of host optimal life-history strategies (e.g. Sheldon and Verhulst 1996). In this chapter, we review the evidence for, and the mechanisms underpinning, a phenomenon termed "density-dependent prophylaxis" (DDP; see Table 1; Wilson and Reeson 1998).

The density-dependent prophylaxis hypothesis argues that because the potential threat posed to organisms by their parasites generally changes as a function of population density, if disease resistance is costly to maintain, then prophylactic investment in disease resistance mechanisms should be phenotypically plastic and individuals should tailor them to meet this predictable threat (Wilson and Reeson 1998). More specifically, because parasite transmission tends to be positively density-dependent, the *per capita* risk of infection generally increases with population density and individuals in crowds are generally under greatest threat from parasites (Anderson and May 1981). As a consequence, it is predicted that organisms will increase their investment in disease resistance mechanisms (immunological, behavioral, chemical and/or physical) as population density increases, and that this will result in a positive relationship between population density and *per capita* parasite resistance. This prediction is particularly interesting because it is counter-intuitive; it is generally assumed that crowding is "stressful" and will lead to an increase in susceptibility to parasites (Steinhaus 1958,1963).

Whilst it is true that parasitism and disease are both more likely to be prevalent under crowded conditions, the DDP hypothesis argues that this is largely a consequence of the nature of the density-dependent transmission process. Thus, as population density increases, and the risk of infection rises, so optimal investment in disease resistance mechanisms increases, dose-dependent *per capita* mortality rate falls, but *overall* mortality due to parasitism may continue to rise. Crucially, however, the rate at which parasite-induced mortality increases with population density will be slower than that observed in the absence of density-dependent prophylaxis (Fig. 1). The DDP hypothesis makes three specific predictions: (i) for a given challenge of parasites, the *per capita* mortality rate will decline as previous experience of crowding increases; (ii) as the degree of crowding increases, so *per capita* investment in disease resistance mechanisms will increase (assuming that resistance increases in proportion to investment levels); and (iii) under field conditions, parasite-induced host mortality will be a

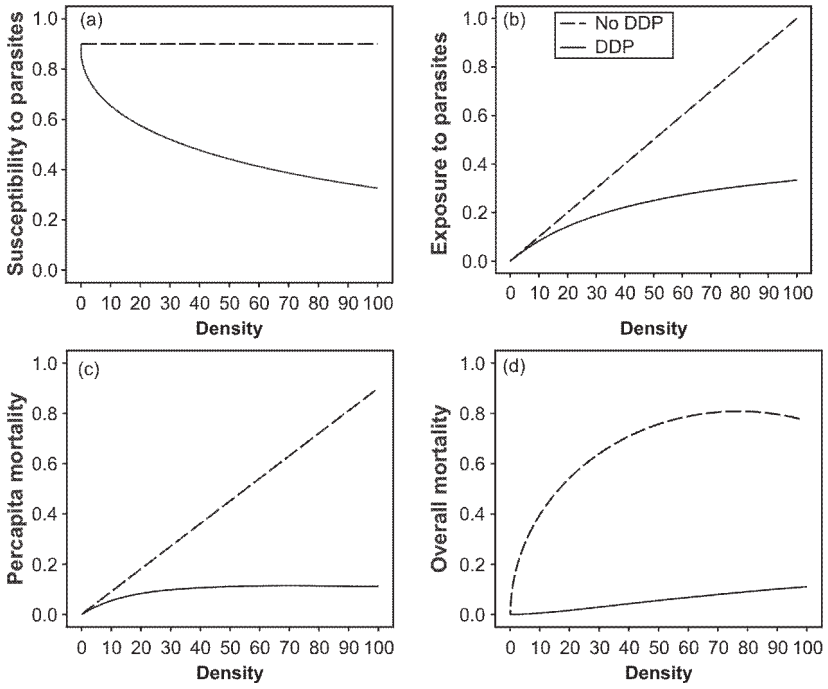

Fig. 1 A graphical representation of the possible effect of (a) density-dependent investment in parasite resistance on (b) exposure to parasites, (c) *per capita* mortality and (d) overall mortality. The dashed lines show the patterns expected in the absence of density-dependent prophylaxis, and the solid lines show those predicted by the DDP hypothesis. Note that the exact patterns depend on the precise functions used to describe DDP and parasite transmission. All scales are arbitrary.

saturating function of host density. Of these three predictions, the third is the weakest because it may also be generated by non-adaptive mechanisms (D'Amico et al. 1996; Dwyer et al. 1997). In all cases, it is assumed that there is no degree of crowding that constrains the optimal decision. However, there may be an upper threshold above which "stresses" limit the options set (e.g. see Goulson and Cory 1995, and below).

The DDP hypothesis emerges from the interaction of life-history theory (e.g., Stearns 1992; Roff 2002) and epidemiological theory (e.g., Anderson and May 1991), and forms part of a growing body of theory generated by the relatively new field of 'ecological immunology' (Sheldon and Verhulst 1996; Wilson 2004). It relies on three important assumptions. First, that parasite transmission is generally positively density-dependent. Second, that potential hosts can alter their phenotype in response to cues associated with

population density. And third, the DDP hypothesis implicitly assumes that parasite defense is costly.

Assumptions

Parasite Transmission is Positively Density-Dependent

Density-dependent transmission of parasites has been a fundamental assumption of most epidemiological models for nearly a century (see review by McCallum et al. 2001). The reasoning is fairly straightforward—as population density increases, so too does the *per capita* risk of an individual encountering an infectious conspecific/cadaver or parasite infective stage. This idea is based on the "mass action" concept; namely that infectious and susceptible individuals within a population behave like the molecules of two chemical reagents in a closed system, moving in space randomly in relation to each other, such that the encounter rate between the two types of molecules is directly proportional to their relative densities. Thus, if the density of susceptible hosts is represented by S, and the density of infected hosts (or other infectious agents) is I, then the number of new infected hosts per unit area per unit time will be βSI, where β is the so-called *transmission coefficient*—a constant representing the probability of a new infection arising *per contact* between a susceptible and infectious host.

Although most epidemiological models have assumed that the transmission rate is dependent on host density, not all parasites are expected to exhibit density-dependent transmission. For some parasites, the contact rate between susceptible and infected individuals is predicted to be independent of host density (i.e., 'frequency-dependent' or 'density-independent') or there may be an asymptotic relationship between contact rate and host density (McCallum et al. 2001). For example, it is often assumed in models of sexually-transmitted diseases that because the number sexual partners of an individual usually depends on its attractiveness and/or the mating system of the species, parasite transmission will be only weakly related to host density (and the number of new infections per unit time and area will approximate $\beta SI/N$, where $N = S + I$).

Although 'mass action' is commonly assumed in epidemiological models, it has only rarely been empirically challenged. In insect-parasite studies, tests of the mass action assumption have generally involved manipulating the density of infectious and/or susceptible hosts and determining the relationship between I and/or S and the transmission coefficient, β (Dwyer 1991).

The constant β can be calculated using a protocol developed by Dwyer (1991), which is derived from the basic Anderson and May (1981) model.

$$\beta = \frac{-1}{I_0 t} \ln\left[1 - \left(\frac{I_t}{S_0}\right)\right] \quad (1)$$

where I_0 is the initial density of infected hosts/cadavers; t = number of days of exposure to the infected hosts/cadavers; I_t is the density of infected hosts at time t; and S_0 is the density of susceptible hosts at the start of the experiment. This calculation assumes that the change in density of infectious hosts over the course of the experiment is negligible, that all infections are lethal, and that there are a negligible number of deaths due to non-parasite causes; all of these assumptions can be tested. If the mass action assumption applies, then β will be independent of both I and S and the rate of acquisition of new infections will increase linearly with the density of infectious and susceptible hosts (e.g. Anderson and May 1981; Dwyer 1991).

Experimental tests of the mass action assumption (reviewed in McCallum et al. 2001) have shown that although parasite transmission usually exhibits some form of positive density-dependence (Fig. 2), in most cases the strict assumption of mass action is violated (and β tends to decline with I and may decline or increase with S; McCallum et al. 2001). Thus, although it is intuitively appealing to assume that the *per capita* risk of becoming infected increases with local population density, and the available evidence is broadly in support of this idea, more studies explicitly testing this assumption are clearly required.

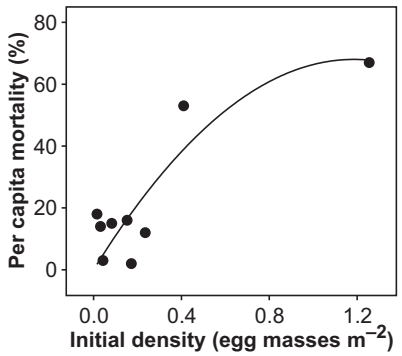

Fig. 2 Relationship between larval density and *per capita* infection rate in the gypsy moth, *Lymantria dispar* (data extracted from Woods and Elkinton 1987). Solid line is a second order polynomial constrained to pass through the origin. As host density increases, so too does the *per capita* risk of infection.

Host Phenotype is Phenotypically Plastic with Respect to Conspecific Population Density

A second assumption underlying the DDP hypothesis is that organisms can alter their phenotype in response to cues associated with population density (and hence the risk of infection). Perhaps more than any other taxonomic group, insects are renowned for their great phenotypic plasticity in response to population density. This is typified by nymphs of the desert locust, *Schistocerca gregaria*, which exist as a green, cryptic form under low density conditions and a conspicuous yellow-and-black form under high density conditions (e.g., Uvarov 1966; Pener 1991; Applebaum and Heifetz 1999) (see Figure 8). Density-dependent phenotypic changes are not restricted to color however; they also include alterations in morphology, physiology, behavior, development and feeding ecology (Applebaum and Heifetz 1999).

When an individual's phenotype is altered in response to perceived changes in the local density of conspecifics, it is usually known as "density-dependent phase polyphenism" (Table 1); or sometimes as "phase polymorphism". However, this latter expression is misleading, since polymorphisms usually refer to genetically-based changes in phenotype, as opposed to those that occur in response to changes in environmental conditions (Table 1) (Chapman 1998). Density-dependent phase polyphenism has been characterized in insects belonging to a wide range of insect taxa, including Lepidoptera, Phasmida, Orthoptera, Coleoptera, Hemiptera and Homoptera. In some phase polyphenic species, the phenotype adapted to low density conditions is referred to as the *solitaria* phase, and that adapted to high density conditions as the *gregaria* phase (e.g. Uvarov 1921, 1966; Iwao 1968). Of course, these are the extreme phenotypes that individuals may adopt under very low or high densities, but intermediate forms are usually common, and may reflect differences in exposure to density-dependent cues or genetic differences in the phenotypic response to density (i.e., genotype x environment interactions). Although density-dependent phase polyphenism has been characterized in many insect species, some of which are manifested in rather extreme color or morphological changes, it is likely that most insect species have the potential to alter their phenotype in response to density-dependent cues, though these may not always be apparent in their visible phenotype (i.e., morphology or behavior). Thus, whilst the DDP hypothesis is most likely to be supported by insect species exhibiting overt density-dependent phase polyphenism, it would not be surprising if supporting evidence was forthcoming from non-phase polyphenic species.

Table 1 Glossary of terms

Density dependent prophylaxis (DDP)	A form of phenotypic plasticity. A phenomenon in which individuals up-regulate their parasite resistance mechanisms under conditions of conspecific crowding to counteract the increased risk of parasitic infection at high densities.
Ecdysteroids	Ecdysone and related hormones. The molting hormone, ecdysone is a steroid produced by the prothoracic glands in juvenile insects. In adults, the gonads produce ecdysteroids, which are involved in gonad maturation (Nijhout 1994).
Encapsulation	The formation of an envelope of cells around an invading organism that is too large to be phagocytosed. The cells become melanized via the action of phenoloxidase (Götz 1986).
Juvenile hormone (JH)	A methyl-ester sesquiterpene hormone produced by the *corpora allata* and involved in many aspects of reproduction and development including metamorphosis, regulation of diapause, phase polyphenism and ovarian development (Nijhout 1994).
LPS	Lipopolysaccharide—a component of bacterial cell walls that can trigger the prophenoloxidase cascade, and is often used in assays of immune function.
Lysozyme	The first antibacterial factor purified from insect hemolymph; its structure is very similar to lysozymes found in chickens. Lysozyme is bactericidal to some gram-positive bacteria, but mostly works in concert with other antibacterial proteins (Boman and Hultmark 1987).
Phase polyphenism	A form of phenotypic plasticity by which a single genotype can produce *distinct* phenotypes depending on the environmental conditions experienced.
Phenoloxidase (PO) and the proPO cascade	Phenoloxidase, a key enzyme in the synthesis of melanin, is stored in the hemolymph as the inactive precursor, prophenoloxidase. The prophenoloxidase cascade is an enzyme cascade that can be triggered by microbial cell wall components, such as peptidoglycan and zymosan, via a serine protease cascade.
Polymorphism	General sense: variation in body form or color within a species (see also *Polyphenism*). Specific sense: genetically-based (as opposed to environmentally regulated) variation in body color or morphology within a species.
Polyphenism	A form of phenotypic plasticity. The occurrence of different phenotypes within a species, where the development of the phenotype is governed by environmental conditions experienced during development, including temperature, humidity and population density (see also *Polymorphism*).
Prophylaxis	Prevention of, or protection from, disease causing agents.

Parasite Defense is Costly

A third assumption implicit in the DDP hypothesis is that the maintenance of parasite defense mechanisms is costly. This is because, if parasite defense

was cost-free, then selection would favor organisms that maintained them at high levels at all times. If parasite defense is costly, then selection will favor individuals that alter their investment in resistance mechanisms to match the perceived risk that they will be required (such as when levels of crowding are high). There is now good evidence from a number of studies indicating that both the maintenance and deployment of parasite resistance mechanisms are costly (see reviews by Lochmiller and Deerenberg 2000; Kraaijeveld et al. 2002; Schmid-Hempel 2003; Wilson 2004).

One of the clearest examples illustrating the costs of maintaining an effective parasite resistance mechanism comes from the fruit fly, *Drosophila melanogaster* L., and two of its parasitoids. Kraaijeveld and Godfray (1997) set up four genetic lines that were selected for resistance to attack by the braconid parasitoid wasp *Asobara tabida* Nees and compared them with four control lines that were not exposed to the parasitoid. They found that selected lines rapidly increased their cellular encapsulation response to parasitoid eggs from 5% at the start of the experiment to greater than 60% after 5 generations of selection. After extensive investigation, they discovered that the main cost of resistance in this system was a decline in the competitive ability of larvae in the selected lines relative to controls when food was in short supply. Similar experiments with the eucoilid wasp, *Leptopilina boulardi* Forster, showed a similarly rapid response to selection, from less than 1% encapsulation at the start of selection to 45% after just 5 generations (Fellowes et al. 1998). Significantly, increased resistance to parasitism by *L. boulardi* was also achieved at the expense of competitive ability when food was limited. Similar experiments in other insect-parasite systems have also indicated that parasite defense is costly to maintain, though these costs may be revealed only under certain environmental conditions (e.g., when animals are food stressed) (see reviews by Schmid-Hempel 2003; Wilson 2004).

Empirical Examples

Density-dependent prophylaxis has been tested and/or observed in a range of insect species, including both phase polyphenic and non-polyphenic species (Table 2). Amongst the phase polyphenic species, most of the examples come from Lepidoptera, Coleoptera and Orthoptera. Amongst the non-polyphenic species, examples are from the Hymenoptera and Isoptera.

Phase Polyphenic Species

Lepidopteran Larvae Pathogen resistance has been examined in relation to phase and/or rearing density in a number of phase-polyphenic,

Table 2 Insect species tested for density-dependent resistance to pathogens and/or immune function

Order	*Species*	*Phase polyphenic*	*Density-dependent resistance to pathogens*	*Density-dependent immune response*
Lepidoptera	*Spodoptera littoralis*	Y	Fungus resistance increases with melanism and rearing density (Wilson et al. 2001)	Cuticular PO increases and antibacterial activity decreases with density (Cotter et al. 2004a) Hemolymph PO, cuticular PO and capsule melanization increase and antibacterial activity decreases with melanism (Cotter et al. 2004a)
Lepidoptera	*Spodoptera exempta*	Y	Virus resistance increases with density (Reeson et al. 1998) Fungus resistance increases with density at low doses but not high (Wilson et al. 2001) Parasitoid resistance increases with melanism (Wilson et al. 2001)	Hemolymph PO, cuticular PO and midgut PO increase with density (Reeson et al. 1998; Wilson et al. 2001)
Lepidoptera	*Mamestra brassicae*	Y	Virus resistance increases with density but falls at very high densities (Goulson and Cory 1995)	
Lepidoptera	*Mythimna separata*	Y	Resistance to fungus increases with melanism (Mitsui and Kunimi 1988) Resistance to virus increases with melanism and rearing density (Kunimi and Yamada 1990)	
Coleoptera	*Tenebrio molitor*	Y	Resistance to fungus increases with melanism (Barnes and Siva-Jothy 2000)	No effect of density on hemolymph PO (Barnes and Siva-Jothy 2000)
Orthoptera	*Locusta migratoria*	Y	Resistance to fungus increases with density (Wilson et al. 2002)	No effect of density on hemolymph PO, antibacterial activity increases with density (Wilson et al. 2002)
Hymenoptera	*Acromyrmex echinatior*	N	Resistance to fungus increases with density (Hughes et al. 2002)	
Isoptera	*Zootermopsis angusticollis*	N	Resistance to fungus increases with density (Rosengaus et al. 1998)	No effect of density on encapsulation (Traniello et al. 2002)

lepidopteran species. In many cases, these two factors can be hard to separate as phenotype and density may be inextricably linked. However, in some species, the typical high-density phenotype can occur at low densities and vice-versa, thus allowing the effects of rearing density and phase to be disentangled.

Mythimna separata Using larvae of the armyworm, *Mythimna (Pseudaletia) separata* Walker, Kunimi and Yamada (1990) examined the relationship between density, color phase and pathogen resistance. Larvae were reared in solitary (1 larva per container) or crowded conditions (2, 5 or 20 larvae per container) from the onset of the second instar until the onset of the fifth instar, at which time a nucleopolyhedrovirus (NPV) was administered orally. They found that susceptibility to NPV decreased with rearing density, such that larvae in the highest density treatment were 20 times more resistant to the virus than those reared solitarily (Fig. 3a). The experiment was then repeated with solitary and crowd-reared (20 larvae per container) treatments, but this time the density experienced by the larvae was switched *after* administration of the virus, giving four treatment groups: solitary-solitary (SS), solitary-crowded (SC), crowded-solitary (CS), and crowded-crowded (CC). LC_{50} values showed that whilst SS larvae were most susceptible and CC most resistant to the virus, the two groups for which the rearing density was switched after viral administration (SC and CS) showed similar, but intermediate, levels of resistance to the other two groups. This highlighted the fact that rearing density both prior to and during infection were important factors in determining an individual's resistance levels. When they injected non-occluded virus directly into the haemocoel (i.e., virus that was not enclosed in a proteinaceous occlusion body), they found that there was no difference between solitary and crowd-reared larvae in their susceptibility, suggesting that resistance mechanisms in the gut may be responsible for the increased resistance shown by crowd-reared larvae.

Density-dependent prophylaxis in this species was reflected not only in resistance to its NPV, but also in resistance to its granulovirus virus (GV) (Kunimi and Yamada 1990) and to the generalist entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson (Mitsui and Kunimi 1988). Relative to gregarious larvae, solitary *M. separata* larvae were about 5 times more susceptible to oral doses of GV (Kunimi and Yamada 1990; Fig. 3b), and twice as susceptible to percutaneous doses of *N. rileyi* conidia (Mitsui and Kunimi 1988; Fig. 3c).

The role of color phase in pathogen resistance was also examined in this species, whilst controlling for rearing density. Although melanism

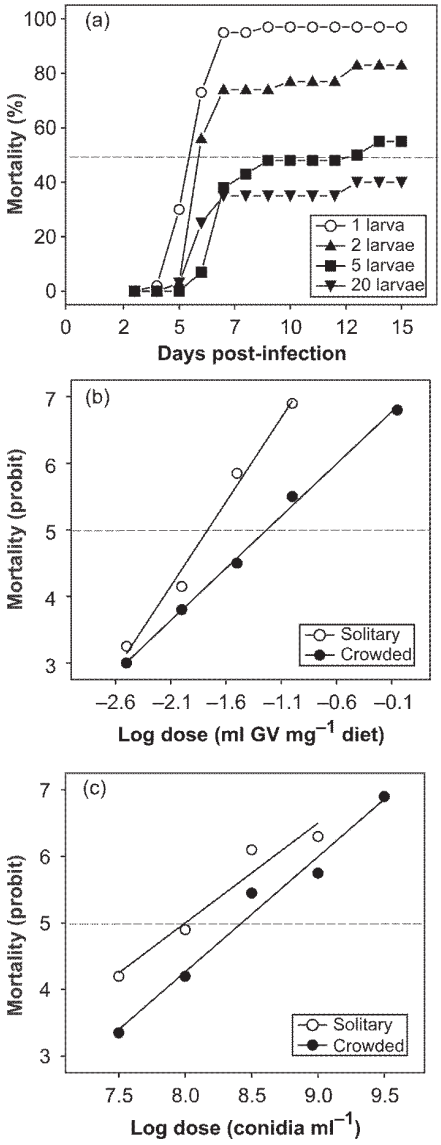

Fig. 3 Relationship between larval density and resistance to parasites in the true armyworm, *Mythimna* (*Pseudaletia*) *separata*. (a) Cumulative mortality for larvae reared at 1, 2, 5 or 20 larvae per container and orally infected with its nucleopolyhedrovirus, NPV (Kunimi and Yamada 1990); (b) Dose-mortality relationship for solitary and crowded larvae orally infected with its granulovirus, GV (Kunimi and Yamada 1990); (c) Dose-mortality relationship for solitary and crowded larvae percutaneously infected with the fungus, *Nomuraea rileyi* (Mitsui and Kunimi 1988). The dashed line in each graph indicates 50% mortality.

generally increases with density, it can occur in low-density animals. Low-density melanic larvae were 5 times more resistant to the entomopathogenic fungus *Nomuraea rileyi* (Mitsui and Kunimi 1988), whilst melanic larvae that occurred at high densities were twice as resistant to an NPV than pale larvae (Kunimi and Yamada 1990).

Spodoptera spp. More recent studies have tried to quantify both pathogen resistance and investment in immune function in relation to phase and rearing density. In the African armyworm, *Spodoptera exempta* Walker (Fig. 4), resistance to its NPV was lowest in non-melanic, solitary-reared larvae (average mortality over a range of viral doses = 70%; LD_{50} = 1,325 OB/larva) and highest in melanic crowd-reared larvae (42%; 14,188 OB/larva), whilst melanic, solitary-reared larvae showed intermediate levels of resistance (59%; 3,082 OB/larva; Fig. 5a; Reeson et al. 1998). Thus, the typical solitary form of *S. exempta* was approximately 10 times more susceptible to NPV than the typical gregarious form.

Investment in hemolymph phenoloxidase, a key enzyme in the production of melanin and an important component of the insect immune system, followed the same patterns, increasing from solitary-reared, non-

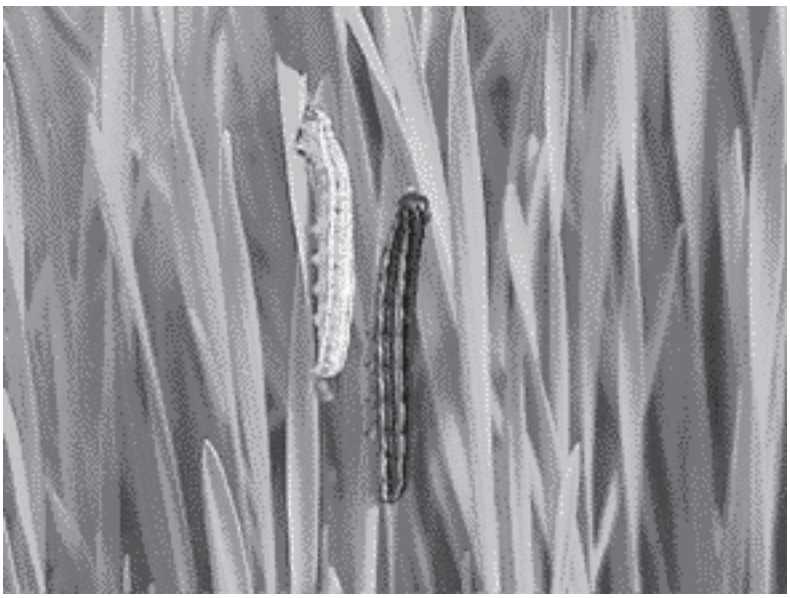

Fig. 4 Cuticular melanism in the African armyworm, *Spodoptera exempta* (Wilson et al. 2001). The two larvae are siblings; the one on the left was reared solitarily, the one on the right in crowded conditions. Photograph Courtesy of Ken Wilson.

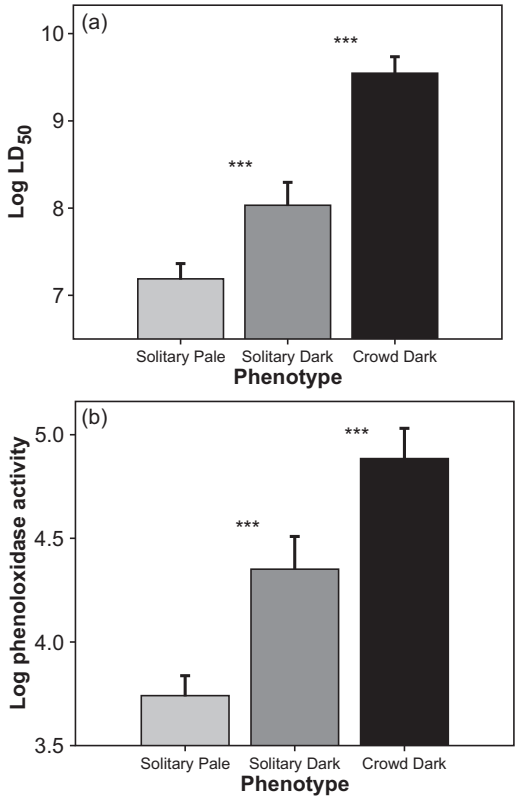

Fig. 5 The relationship between larval density/phase and (a) resistance to a nucleopolyhedrovirus, measured as the dose of virus required to kill 50% of the larvae, LD_{50} (Reeson et al. 1998), (b) immune function measured as hemolymph phenoloxidase activity (Wilson et al. 2001). Bars show means ± standard errors.

melanic larvae to crowd-reared, melanic larvae (Fig. 5b; Reeson et al. 1998). A further examination of parasite resistance and immune function in this species found that crowd-reared, melanic larvae were more resistant both to the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (at low doses, but not high), and to the ectoparasitoid, *Euplectrus laphygmae* Ferrière, than solitary-reared, non-melanic larvae (Wilson et al. 2001). Moreover, investment in phenoloxidase in the midgut and cuticle, important sites of entry for many parasites and pathogens, increased with increasing melanism (Fig. 6a), suggesting that it could be the phenoloxidase in these sites that is providing the resistance (Fig. 6b).

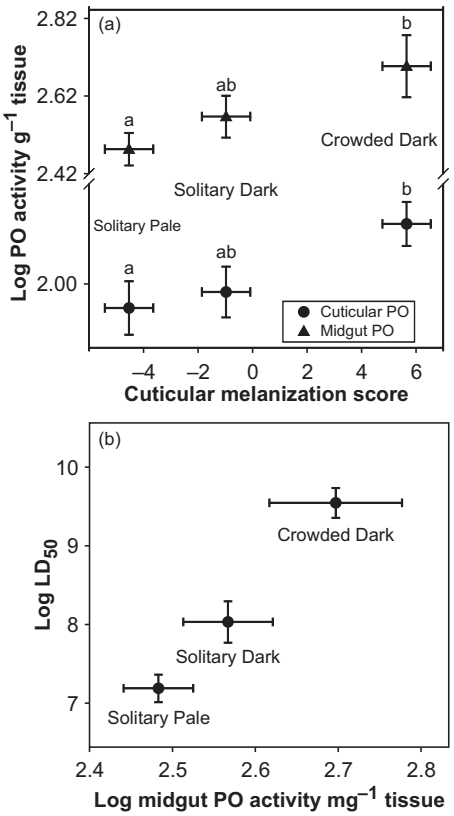

Fig. 6 Cuticular melanism, phenoloxidase activity and resistance to nucleopolyhedrovirus in the African armyworm, *Spodoptera exempta* (Wilson et al. 2001). (a) Cuticlar melanization is associated with increasing levels of phenoloxidase activity in the cuticle and midgut; (b) elevated levels of phenoloxidase activity in the midgut (and elsewhere) are associated with enhanced resistance to nucleopolyhedrovirus, as measured by LD_{50} (for details see legend to Fig. 5a). In each graph, means $\pm$ standard errors are shown.

The only study to examine the relationship between phase, rearing density and a suite of immune system components found surprising results. Using larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval, it was shown that, as predicted by the DDP hypothesis, levels of phenoloxidase activity in the hemolymph and cuticle were higher in melanic than non-melanic larvae, as was melanization of an artificial parasite (Figs. 7a-c). However, (lysozyme-like) antimicrobial activity levels in the hemolymph were highest in non-melanic and solitary-reared larvae (Fig. 7d), suggesting a potential trade-off in the insect immune system (Cotter et al. 2004a).

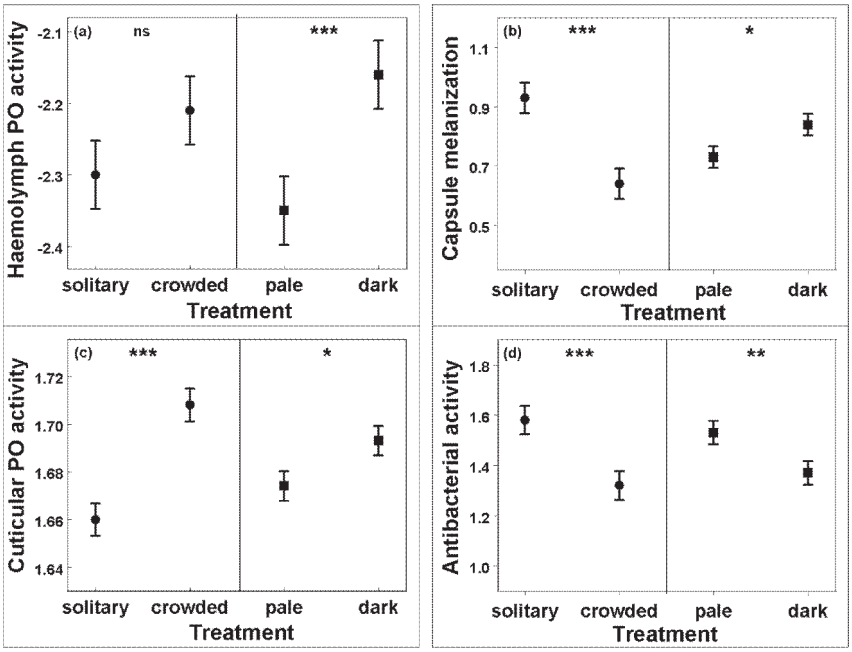

Fig. 7 Effect of color and larval density on immune function parameters in larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. Larvae were reared in solitary or crowded conditions and scored for color: pale or dark. The figures show mean (+/- SE) levels of investment in four different immune parameters: (a) hemolymph phenoloxidase activity, (b) cuticular phenoloxidase activity, (c) capsule melanization of a foreign implant, and (d) lysozyme-like antibacterial activity (Cotter et al. 2004a).

Other Lepidopteran species Goulson and Cory (1995) examined the relationship between pathogen resistance and rearing density in the cabbage moth, *Mamestra brassicae* L. and found that resistance to NPV (and larval melanism) increased with rearing density, although in this case larvae that were reared at exceptionally high densities experienced a decrease in resistance, suggesting that under very crowded conditions adaptive responses to density may break down.

Mealworm beetles Pathogen resistance was examined in the weakly phase-polyphenic coleopteran *Tenebrio molitor* L. Adult mealworm beetles range in color from tan to black, with melanic (black) beetles being more common at high densities. Melanic *T. molitor* were up to three times more resistant to the entomopathogenic fungus *Metarhizium anisopliae* than the paler beetles (Barnes and Siva-Jothy 2000). However, in this species, there was no association between hemolymph phenoloxidase activity and rearing

density. Unfortunately, the correlation between phenoloxidase activity and melanism was not determined directly and phenoloxidase activity was not measured in the cuticle, so the basis for the increased resistance of melanic beetles is not clear (Barnes and Siva-Jothy 2000).

Desert locusts The desert locust (*Schistocerca gregaria* Forskal) is the archetypal phase polyphenic species. Therefore, if the DDP hypothesis is to be credible, then it should apply to this species. Wilson et al. (2002) tested the DDP hypothesis using the desert locust and found that locusts reared under crowded conditions were significantly more resistant than solitary-reared locusts to the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikov) Sorokin *var acridum*, a key natural disease of acridids and an important agent in locust and grasshopper biocontrol. After accounting for body mass differences between the phases, the relative daily mortality risk for the *solitaria* phase locusts was nearly twice that of *gregaria* phase conspecifics (mean = 1.76; 95% confidence interval = 1.35–2.31; Fig. 8). The enhanced pathogen resistance observed in the crowded locusts was associated with significantly elevated antimicrobial (lysozyme-like) activity, a marginally greater total hemocyte count, and non-significant differences in hemolymph phenoloxidase activity and cellular

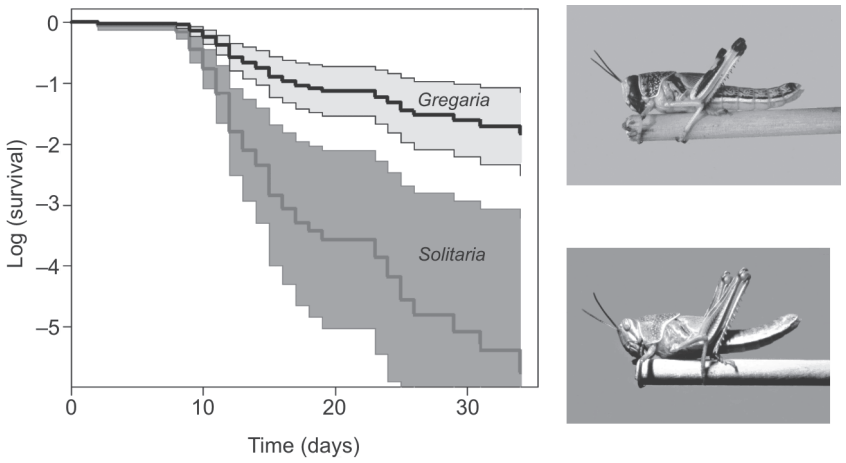

Fig. 8 Density-dependent changes in the desert locust, *Schistocerca gregaria*. Log-survival curves for *solitaria* and *gregaria* phase locusts infected with the *Metarhizium anisopliae* var *acridum* (Wilson et al. 2001). The two bold lines show the fitted values from the Cox's Proportional Hazard Model and the narrow lines and shading represent the 95% confidence intervals. Photographs courtesy of Stephen J. Simpson.

encapsulation response. Thus, as predicted by the DDP hypothesis, the high-density form of the desert locust exhibits significantly greater pathogen resistance than the solitary form. However, the precise immunological and/or physical mechanisms underpinning this observation have yet to be determined.

Non-phase Polyphenic Species

Leafcutting ants. Studies examining the effects of density on pathogen resistance in non-phase polyphenic species are rare. However, a recent study found that after inoculating *Acromyrmex echinatior* Forel workers with the fungus *Metarhizium anisopliae*, those that were kept with groups of nestmates showed reduced mortality compared to those kept in isolation (Hughes et al. 2002). It was proposed that this was due to a combination of hygienic allogrooming and increased antibacterial secretions stimulated by the presence of fungal spores. Previous work has shown that the prevalence of allogrooming increases with colony size across ant species (Schmid-Hempel 1998).

Termites A similar study was conducted using nymphs of the dampwood termite, *Zootermopsis angusticollis* Hagen (Rosengaus et al. 1998). After exposure to spores of the entomopathogenic fungus, *Metarhizium anisopliae*, nymphs were maintained individually or in groups of 10 or 25. A strong effect of grouping was apparent with nymphs that were kept in groups showing a 90% reduction in their daily mortality risk compared to those kept in isolation. By monitoring the behavior of nymphs that had been exposed to spores, it was shown that the frequency of allogrooming was greatly increased in those exposed to the fungus compared to controls. However, the frequency of self-grooming did not increase, either in nymphs maintained in groups or in isolation, suggesting that grooming by nestmates is necessary to remove fungal spores and so reduce the risk of infection.

A more recent study using the same species examined further adaptations to reduce the spread of disease at high densities (Traniello et al. 2002). Termites respond to a non-lethal dose of a bacterial or fungal pathogen by improving their physiological response to a later infection, i.e. they can become immunized against particular pathogens (Rosengaus et al. 1999). By grouping naive and immunized termite nymphs prior to a challenge with a lethal dose of fungal spores, it was found that there was a "social transfer" of immunity from immunized to naïve nymphs. Nymphs that had been immunized by this social transfer displayed a 13-fold reduction in susceptibility compared to controls (Traniello et al. 2002). The

mechanisms underlying this social transfer of immunity are unclear. Naïve nymphs may be exposed to a sub-lethal dose of spores during allogrooming of immunized individuals or there could be a transfer of immune factors during trophallactic exchanges between naïve and immunized individuals (Traniello et al. 2002). However, it appears that nestmate density *per se* does not affect susceptibility to fungal pathogens in this species. A study examining the effects of high versus low density on susceptibility to *M. anisopliae* found no significant difference in mortality between the treatments (Pie et al., 2005). Furthermore, an examination of the encapsulation response found no difference between solitary and grouped termites, suggesting that it is not an up-regulation of immunity in response to high density that causes the reduction in mortality in grouped versus solitary termites (Traniello et al. 2002).

Social insects naturally occur at high densities and so behavioral mechanisms such as those described above may reduce the risk of parasitism in groups and as such fall within the remit of the DDP hypothesis. However, the DDP hypothesis has yet to be tested in a species that undergoes strong fluctuations in population density and does not display density-dependent phase polyphenism.

Field Experiments

The non-linearity observed in the larval density-infection rate plots generated from field experiments is consistent with the DDP hypothesis (e.g. Woods and Elkinton 1987; D'Amico et al. 1996; Dwyer et al. 1997; Fig. 2). However, to our knowledge, there has so far been just one direct field-based test of the DDP hypothesis. Reeson et al. (2000) reared larvae of the African armyworm (*S. exempta*) in the laboratory under either solitary or crowded conditions, and when they reached the fourth instar they were introduced into 1 m^3 field cages containing 9 maize plants. The larvae were introduced to the plants at a density of one, three or nine larvae per plant. A few days prior to this, the maize plants were 'seeded' with two larvae that had been lethally-infected with nucleopolyhedrovirus (NPV). Thus, the introduced larvae were exposed to infectious cadavers for several days before being reclaimed and reared again in the laboratory under solitary conditions until pupation or death.

Using this experimental design, it was possible to estimate the effect of rearing density (solitary versus crowded) and exposure density (one, three or nine larvae per plant) on both the *per capita* mortality rate and the viral transmission parameter, β (see Dwyer 1991, and Eq. 1 above). As predicted

by the DDP hypothesis, Reeson and colleagues found that both the mortality rate and the viral transmission parameter were significantly lower for larvae that were reared under crowded conditions prior to exposure to the virus than for larvae that were reared under solitary conditions (Fig. 9). Thus, the mass action assumption was not upheld for *S. exempta* and its NPV. Local density during the experiment (i.e., the number of larvae per plant) had little effect on viral transmission (Reeson et al. 2000). It is important to realize here that the transmission parameter, β, comprised two components, namely the rate at which the host encounters parasites (i.e., the contact rate) and the rate at which contact between hosts and parasites results in infection (i.e., host

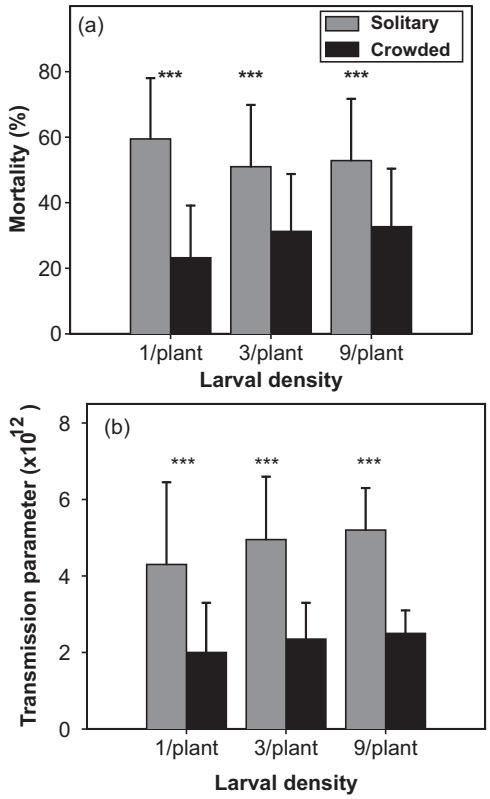

Fig. 9 Relationship between larval density and (a) average mortality and (b) the transmission parameter, for larvae of the African armyworm, *Spodoptera exempta,* exposed to its nucleopolydrovirus (NPV) under field conditions. Mortality and viral transmission were greatest for larvae reared under solitary, rather than crowded conditions until the start of the 4th instar, but did not vary significantly with the density of larvae during the few days that the larvae were exposed to the virus (Reeson et al. 2000). Means $\pm$ standard errors are shown.

susceptibility). The experiment described here indicates that differences in host susceptibility generated by DDP may be large enough to produce density-dependent changes in β.

Mechanisms

Sensory Systems Underlying Plasticity: Perception of Density

A key question in the study of DDP, and of phase polyphenism in general, is the relative importance of different mechanisms for the perception of population density. This issue has been most thoroughly addressed in studies of the desert locust, *Schistocerca gregaria*. Experimental studies have shown that locust phase determination is governed by the combined effects of genetic, maternal and environmental effects. For example, having a crowd-reared mother, a crowd-reared father, parents which were crowded only for the period of mating and oviposition, or being reared from birth in a crowd, all had a marked gregarizing effect on the behavior and color of desert locust hatchlings (Islam et al. 1994). However, the most important factor determining locust phase was the individual's perception of local population density.

When desert locust nymphs are switched between low and high-density conditions, different aspects of the suite of phase characteristics change at different rates. Changes in metabolism and behavior occur within just 1–4 hours of the switch, whereas color and morphological changes can occur only following molting to subsequent instars (e.g. Roessingh and Simpson 1994; Applebaum and Heifetz 1999). Olfactory, visual and tactile stimuli all play a role in determining whether individuals develop into *solitaria* or *gregaria* phase locusts (Uvarov 1966). In an elegant series of experiments, Roessingh et al. (1998) examined the relative importance of these different stimuli in determining behavioral phase transition in fifth-instar nymphs of the desert locust. They found that whilst tactile stimulation (by rolling paper spheres) was highly gregarizing, and olfactory and visual stimuli together caused partial behavioral gregarization, visual and olfactory stimulation provided alone were only weakly gregarizing, if at all. A number of other studies on lepidopteran species also appear to support the notion that tactile stimulation is the key trigger for density-dependent phase changes (e.g. Drooz 1966; Sasakawa 1973; Kazimirova 1992; Gunn 1998).

In subsequent experiments on the desert locust, Simpson et al. (2001) observed that a significant switch from solitarious to gregarious behavior occurred when the outer face of a hind femur was stimulated (by a paint

brush), but mechanical stimulation of 10 other body regions (including other parts of the legs, the abdomen, thorax, antennae and mouthparts) did not result in significant behavioral changes (Fig. 10). Thus, it appears that stimulation of mechanoreceptors on the hind femora is the most important factor determining behavioral phase change in desert locusts, probably because this part of the body is least likely to be self-stimulated or stimulated during normal behavioral activities, such as feeding. It remains to be established whether this also serves as the trigger for the up-regulation of parasite resistance mechanisms in this same species (Wilson et al. 2002, see above).

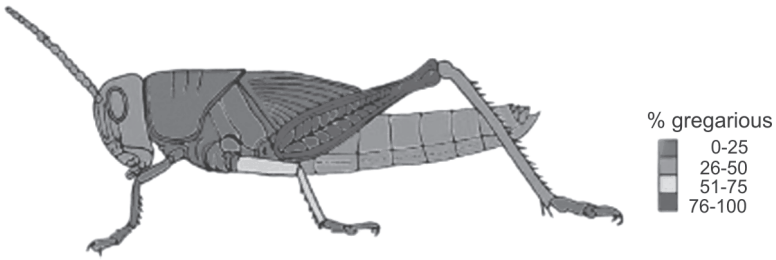

Fig. 10 Effect of tactile stimulation on the propensity to change from *solitaria* to *gregaria*-phase behavior in the desert locust, *Schistocerca gregaria*. The different colors indicate the probability of being classified as a *gregaria* phase nymph on the basis of behavior following 4 h of mechano-stimulation to different body regions (Simpson et al. 1998).

Genetics of Phase and Density-Dependent Prophylaxis

Surprisingly little is known about the genetic basis of phase transition. However, in the desert locust *Schistocerca gregaria*, a recent study examined phase-specific gene expression in the brains of solitary and gregarious adults using differential display and reverse transcriptase PCR (Rahman et al. 2003b). This revealed one solitary-specific gene (SSG) and one gregarious-specific gene (GSG). Although the SSG could not be identified, the GSG showed homology with the SPARC gene (Secreted Protein Acidic, Rich in Cysteine) known to regulate growth factors and cell adhesion in vertebrates (Brekken and Sage 2000). An examination of the phase-dependent peptide profile in desert locusts revealed differentially-expressed serine protease inhibitors belonging to the pacifastin peptide family (Rahman et al. 2002). Quantification of the transcription levels of the precursors of these peptides (SGPP-1–3) in solitary and crowd-reared locusts revealed higher expression in crowd-reared than solitary-reared

individuals (Simonet et al. 2004). Furthermore, an immunoregulatory role for these peptides has been posited; injection of locusts with fungal elicitors of the immune response resulted in changes in the abundances of the SGPP transcript (Simonet et al. 2004).

A more detailed study examined ESTs isolated from the head, gut and hind leg of solitary and gregarious *L. migratoria* (Kang et al. 2004). Kang et al. found a number of genes which were differentially expressed, both between the phases and between the different tissue types. A number of muscle-related genes from the hind-leg library were up-regulated in solitary hoppers, which is consistent with the observation that solitary hoppers tend to have longer hind legs and greater jumping ability than gregarious hoppers. Over 100 genes from the midgut library were uniquely expressed in solitary hoppers, the majority of which were metabolism-related. The ESTs isolated from the head showed striking differences between phases in the JHPH superfamily of genes, which includes juvenile hormone binding protein, haemocyanin and phenoloxidase amongst others. Three JHPH families were identified based on sequence similarity, all of which were highly expressed in the head of gregarious hoppers and in the hind leg of solitary hoppers. JHPH1 was up-regulated in gregarious hoppers whilst JHPH3 was up-regulated in solitary hoppers; a number of genes from the JHPH2 family were either expressed in the gregarious head or in the solitary head but not in both. Further identification of these genes and their possible function should provide a valuable insight into the genetic control of phase change in this species.

In Lepidoptera, phase-polyphenism is mainly determined by rearing density, but it also has a genetic component (Tojo 1991; Goulson 1994; Cotter et al. 2004a). However, to date there has been just one study examining the quantitative genetics of phase and immunity. Using larvae of the Egyptian armyworm, *Spodoptera littoralis,* Cotter et al. (2004b) calculated heritability estimates for several immune function and life-history traits and the genetic and phenotypic correlations between them (Table 3). Based on the correlations between traits, notably cuticular melanization, it was possible to predict theoretical life-history trajectories based on color-phase, i.e., pale or dark larvae (Table 4). Pale larvae are characterized by a slow larval development rate and short adult longevity, both traits known to be common to solitary phase Lepidoptera. An important discovery was that whilst hemocyte density was positively genetically correlated with phenoloxidase activity, it was negatively genetically correlated with lysozyme-like antimicrobial activity (Table 3), providing further evidence for a potential trade-off in the insect immune system identified in a previous phenotypic

Table 3 Heritabilities and genetic and phenotypic correlations between traits in solitary-reared *Spodoptera littoralis* larvae

	Larval development rate	*Pupal weight*	*Pupal development rate*	*Adult longevity*	*Cuticular melanization*	*Hemolymph PO activity*	*Antibacterial activity*	*Hemocyte density*
Larval development rate	**0.42** ***	0.21 ***	0.09 *	–0.11 *	0.04 ns	–0.10 *	0.08 ns	0.07 ns
Pupal weight	0.24 ***	**0.49** ***	–0.09 *	0.11 *	–0.02 ns	0.10 *	–0.08 ns	–0.07 ns
Pupal development rate	0.20 *	–0.34 ***	**0.20** **	0.02 ns	–0.05 ns	–0.05 ns	0.08 ns	–0.06 ns
Adult longevity	–0.08 ns	0.41 ***	–0.49 ***	**0.22** **	–0.10 *	0.06 ns	0.05 ns	0.02 ns
Cuticular melanization	0.18 ***	0.00 ns	–0.43 ***	–0.22 ns	**0.36** ***	0.04 ns	–0.01 ns	0.09 *
Hemolymph PO activity	–0.05 ns	0.41 ***	–0.38 ***	0.56 ***	–0.08 ns	**0.65** ***	0.10 *	0.23 ***
Antibacterial activity	–0.29 ***	0.01 ns	0.35 ***	–0.04 ns	–0.06 ns	0.01 ns	**0.63** ***	0.07 ns
Hemocyte density	–0.04 ns	–0.17 **	–0.66 ***	0.04 ns	0.55 ***	0.21 *	–0.23 *	**0.36** ***

Values on the leading diagonal indicate the narrow-sense heritability estimates (after accounting for any significant maternal effects); values above the diagonal show (Pearson's) phenotypic correlations between traits; and values below show genetic correlations. Significance levels were determined with t-tests: ns $p < 0.05$, * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$. Modified after Cotter et al. (2004b)

Table 4 Theoretical life-history trajectories for pale- or dark-phase larvae

Trait	*Pale*	*Dark*
Life-history traits		
Larval development rate	Slow larval development	Fast larval development
Pupal weight	*Low pupal weight*	*High pupal weight*
Pupal development rate	Fast pupal development	Slow pupal development
Adult longevity	*Short adult lifespan*	*Long adult lifespan*
Immune function traits		
Hemolymph PO activity	*Low PO*	*High PO*
Antibacterial activity	*High lysozyme*	*Low lysozyme*
Haemocyte density	Low HC	High HC

Theoretical life-history trajectories were predicted from the point of view of color-phase, i.e. pale or dark larvae, based on the genetic correlations between traits (Table 3). Traits in italics are not directly genetically correlated with cuticular melanization and so the correlations were inferred from relationships with other traits.

study (Cotter et al. 2004a). In terms of the DDP hypothesis, this suggests that insects could not simultaneously up-regulate all components of the immune system in response to crowding, but may up-regulate hemocyte density and phenoloxidase activity at the expense of lytic activity (Table 4). This may represent an important cost of DDP to this insect.

Hormonal Regulation Underlying Plasticity of Phase and Immune Function

As DDP is predicted to occur most strongly in phase polyphenic species, an understanding of the hormonal regulation of phase transition and immune function may elucidate the mechanisms of increased parasite resistance observed in crowded phase individuals.

Phase transition Although the hormonal regulation of phase polyphenism is far from clear, there is compelling evidence, particularly from studies on locusts, that juvenile hormone (JH) plays a key role. The solitary phase of many phase polyphenic Lepidoptera and locusts have high JH titres compared to the gregarious phase (Yagi and Kuramochi 1976; Nijhout and Wheeler 1982). High JH titres are associated with a lack of melanization of the cuticle, with solitary phases tending towards pale or cryptic coloration (Ikemoto 1983; Fescemyer and Hammond 1988). Indeed, exogenous application of JH to gregarious-phase locusts results in a reversion to the green coloration typical of solitary-phase locusts (Pener 1991). Furthermore, as JH is involved in regulation of the molting cycle, high titres usually result

in longer juvenile development times (Yagi and Kuramochi 1976; Fescemyer and Hammond 1988), a common characteristic of solitary phase Lepidoptera.

Low JH titres are also associated with migration (Nijhout 1994); gregarious adults of many species tend to have larger lipid reserves than solitaries and have a greater propensity to migrate (Iwao 1968; Gunn and Gatehouse 1993). Solitary and gregarious phase locusts display a number of phase-specific polypeptides of unknown function in their hemolymph (Wedekind-Hirschberger et al. 1999; Clynen et al. 2002; Rahman et al. 2002; Rahman et al. 2003a). Treatment of gregarious adult locusts with a single dose of the JH analogue, Fenoxycarb, resulted in suppression of nine of the 17 *gregaria*-specific polypeptides and expression of two of the three *solitaria*-specific polypeptides (Wedekind-Hirschberger et al. 1999). Studies such as these underline the importance of JH in phase transition; however it is clear that JH alone is not responsible for the myriad of morphological, physiological and behavioral changes brought on by high densities.

The role of ecdysteroids in phase-transition has also been investigated. The ecdysteroid content of the eggs produced by gregarious desert locust females is significantly higher than in eggs produced by solitaries (Tawfik et al. 1999). This trend is maintained in newly hatched larvae with gregarious larvae having five times the ecdysteroid content of solitaries (Tawfik et al. 1999). However, during larval development, *solitaria* larvae show a higher peak ecdysteroid titer than *gregaria* larvae, though the duration of the hormonal surge is longer in gregarious larvae (Tawfik and Sehnal 2003). Solitary adults also show higher hemolymph ecdysteroid titers than gregarious adults; it may be that low titers stimulate, whilst high titers suppress the production of aggregation pheromones (Tawfik and Sehnal 2003).

Biogenic amines may play a role in density-dependent phase change. Rogers et al (2004) examined the changes in potential neurotransmitters/neuromodulators in the central nervous system of the desert locust, *Schistocerca gregaria* across several stages of gregarization. Crowding of solitarious locusts typically resulted in a rapid decrease in the neurochemicals measured with the exception of serotonin levels in the thoracic ganglia which increased nine-fold during the first four hours of crowding. Increased serotonin levels may be linked to the sensitivity of receptors on the hind leg to mechanical stimulation during this timeframe.

Certain neurosecretory peptide hormones have also been implicated in the regulation of density-dependent color change. Melanization and Reddish Coloration Hormone (MRCH) is responsible for cuticular

melanization in a number of lepidopteran species (Ogura 1975; Matsumoto 1981; Matsumoto et al. 1984; Altstein et al. 1994). A neurohormone isolated from the locust *Locusta migratoria* known as Dark Color Inducing Neurohormone (DCIH) or [His^7]-Corazonin, and the related neurohormone, Corazonin, were found to induce melanization in a number of orthopteran species (Tanaka 2000; Yerushalmi and Pener 2001). Interestingly, Corazonin, which appears to be highly conserved throughout the Insecta, has recently been shown to induce the release of ecdysis-triggering hormones in *Manduca sexta* and *Bombyx mori* (Zitnan D. 2002), suggesting a potential interaction with ecdysone. Furthermore, as neurohormones can stimulate or inhibit JH production, it is possible that there is an interaction between the neurohormones responsible for melanization in different insect species and JH, though this remains to be investigated.

Immune function To date, there have been few studies investigating the hormonal regulation of immune function in insects. However, recently, mediation of the encapsulation response by JH was investigated using the Egyptian cotton leafworm, *Spodoptera littoralis* and its parasitoid *Microplitis rufiventris* Kok. (Khafagi and Hegazi 2001). Application of JH reduced the encapsulation response of *S. littoralis* larvae to *M. rufiventris*, with higher doses showing a stronger effect. Application of anti-JH compounds to parasitised *S. littoralis* larvae resulted in an increased encapsulation rate and enhanced melanization of the capsules, suggesting a possible interaction with phenoloxidase (PO), a key enzyme in the synthesis of melanin.

The link between JH and PO activity is further strengthened by a study showing that JH could inhibit PO activity in *Tenebrio molitor* (Rolff and Siva-Jothy 2002). JH produced by mated adults caused a down-regulation of PO activity, though whether this was direct or indirect is unclear. Using the same species, Rantala et al. (2003) showed that females were more attracted to the pheromones produced by males that had had their JH levels experimentally increased. The same males showed reduced PO levels and encapsulation ability, whilst lysozyme levels were unaffected by the treatment.

There have been several reports of parasitoids depressing PO activity in their hosts (e.g. Kitano et al. 1990; Beck et al. 2000; Shelby et al. 2000; Asgari et al. 2003). Teratocytes, cells that originate from the membrane of parasitoid eggs, have been shown to reduce JH esterase and ecdysone titres in *H. virescens* (Zhang et al. 1992; Dong et al. 1996). The subsequent increase in circulating JH levels may result in a reduced immune response to the

parasitoid. However, decreased ecdysone titres may also play a role in inhibiting the immune response.

In *Drosophila*, the application of ecdysone increased the phagocytic activity of hemocytes *in vitro*, and up-regulated the expression of inducible antimicrobial peptides such as diptericin and drosomysin (Dimarcq et al. 1997). Ecdysone also caused the induction of genes expressing membrane receptors, which are involved in the recognition of microorganisms. In the yellow fever mosquito, *Anopheles gambiae* Giles, an ecdysone receptor site has been identified in the pro-PO 1 gene (Ahmed et al. 1999). Ecdysone up-regulated prophenoloxidase two hours after application and levels increased up to 24 hours later before returning to normal. Furthermore, in ecdysone-deficient *Drosophila*, the encapsulation response is severely compromised, suggesting a key role for ecdysone in this important immune response (Sorrentino et al. 2002).

To date, the evidence seems to point to either ecdysone-mediated *up-regulation* of immune function, or JH-mediated *inhibition* of immune function. However, it is unlikely that these two mechanisms are mutually exclusive. It is commonly accepted that JH can inhibit the ecdysteroid-induced expression of certain genes, such as those involved in metamorphosis (Nijhout 1994) and oogenesis (Soller et al. 1999). It is therefore possible that JH could inhibit the ecdysteroid-mediated expression of certain genes relating to immune function. This would provide an explanation for JH-induced color change, as it is ecdysone that is responsible for cuticular melanization (Curtis et al. 1984; Nijhout 1994). Furthermore, in JH-deficient *Manduca sexta* larvae, levels of dopa decarboxylase (DDC), an enzyme that plays a role in cuticular melanization, were found to be twice those found in control larvae (Hiruma and Riddiford 1993). It could be JH-mediated inhibition of PO expression by ecdysone that is responsible for the reduced PO activity in mated *Tenebrio molitor*. Mating was also found to reduce antibacterial activity in *Drosophila* males, measured as the clearance of *E. coli* from the hemocoel (McKean and Nunney 2001). Again, if mated *Drosophila* males have increased JH titres and this inhibits the ecdysone-mediated regulation of phagocytosis and the expression of inducible antimicrobial peptides such as diptericin and drosomysin, we would expect these males to have reduced antibacterial activity.

It seems that there is a clear relationship between the hormonal regulation of phase transition and immune function in insects. The high-density phase of many orthopteran and lepidopteran species is characterized by high JH titres, which are linked, directly or indirectly, to a number of the

physiological and behavioral traits typical of the phenotype. Similarly, many immune function traits are either inhibited by JH or up-regulated by ecdysteroids. In terms of the DDP hypothesis, it may be that increased parasite resistance is more likely to occur in phase-polyphenic species simply as a by-product of the hormonal regulation of phase. However, it is also possible that selection for increased parasite resistance in crowds preceded the other behavioral and physiological changes associated with phase polyphenism.

Population Level Responses

Inducible, Constitutive and Density-Dependent Defenses

A key question relating to density-dependent prophylaxis is under what circumstances is its adoption favored? Defense mechanisms may be broadly defined as either inducible (plastic) or constitutive. *Inducible defenses* are those that become fully active only after some time delay (for example, it may require time for the fat body to synthesize enough antibacterial peptides to be effective). Whilst inducing these defense mechanisms may be costly, the maintenance of the ability to respond is likely to be relatively cost-free. On the other hand, *constitutive defenses* (such as those associated with the prophenoloxidase enzyme cascade) are effective immediately, but the host must pay the cost of their maintenance even if there is no parasite attack (see Lochmiller and Deerenberg 2000; Kraaijeveld et al. 2002; Schmid-Hempel 2003; Wilson 2004, for reviews of the costs of immune defense). Thus, in the absence of parasites, inducible defense is cheaper than constitutive defense. Shudo and Iwasa (2001) constructed a theoretical model to examine the circumstances favoring the evolution of inducible defense versus constitutive defense versus a combined inducible and constitutive defense. They concluded that constitutive defense is favored over inducible defense when it is relatively effective and cheap; when the initial parasite abundance is large and its growth rate is high; when the difference in the time delay between inducible and constitutive defenses is large; and when the parasite is virulent and frequently attacks. Adopting both constitutive and inducible defense is optimal if the parasite is highly virulent (abundant, high growth rate, causes severe damage) and if the inducible defense is more cost-effective than constitutive defense.

Density-dependent prophylaxis is inducible in response to population density, but it is a constitutive defense in the sense that, once adopted, it is maintained even in the absence of parasites. However, these maintenance

costs are modulated by the perceived risk of them needing to be deployed (i.e. costs are assumed to increase with increasing population density). Thus, DDP represents an alternative defense strategy to purely constitutive or inducible defenses. The circumstances favoring DDP over these alternatives have yet to be formally modeled. However, it seems likely that the adoption of DDP will be favored when: population density fluctuates markedly between generations and reliably predicts infection risk; when the error associated with the perception of population density is low; when the parasite is virulent; when the delay in inducible defenses is long; and when density-dependent investment in defenses is cheaper than density-independent constitutive defense.

Population Dynamic Consequences of Density-Dependent Prophylaxis

As well as affecting the life-histories of the individuals practicing it, density-dependent prophylaxis may also impact on the stability of the host-parasite interaction. White and Wilson (1999) constructed a series of models to examine the impact of DDP on the dynamics of a simple discrete-time host-parasite model in which individuals were born either resistant or susceptible to the parasite (modeled as a micro-parasite or pathogen), and the proportion of individuals born into each host type was potentially density-dependent (characterizing DDP). They found that, under some circumstances, inclusion of a density-dependent resistant class of hosts (i.e. those practicing DDP) might stabilize inherently unstable host-parasite interactions, though greatest regulation was achieved when parasite resistance was density-independent. The stabilizing effect of DDP is enhanced when there is a cost to parasite resistance. Interestingly, the inclusion of DDP in the model favors bi-stable dynamics, in which the final outcome is determined by the initial conditions for the model and either the parasite is driven to extinction or the density-dependent resistant host population grows exponentially. Insect outbreaks, like those common to many phase polyphenic species, are observed in this model only when density-dependent resistance carries no costs, suggesting that the costs of resistance may be trivial in these species (see Discussion).

Density-Dependent Prophylaxis and Biocontrol Strategies

There is substantial interest in reducing reliance on chemical insecticides for the control of insect pests and adopting more environmentally sustainable technologies (Lomer et al. 2001). As a result, dozens of biopesticides have

been, or are being developed, including some to be used against insect species that exhibit DDP (e.g. Cherry et al. 1997; Lomer et al. 2001; Thomas et al. 2001). Ongoing studies are developing an isolate of a nucleopolyhedrovirus against the African armyworm (*S. exempta*) in eastern Africa (Cherry et al. 1997), and biopesticides based on isolates of *M. anisopliae* var *acridum* have been developed for the control of the desert locust (*S. gregaria*) and other acridids throughout Africa, Australia and parts of Europe and Central/Southern America (Lomer et al. 2001; Thomas et al. 2001).

The implications of DDP for these new biopesticides remain to be seen. Certainly, increased transmission efficiency among low-density hosts may allow parasites to persist in low-density host populations better than expected, while high-density populations may be more resistant than expected to invasion by parasites. Thus, counter-intuitively, it is possible that the efficacy and economics of biocontrol strategies may be improved by targeting low-density populations, as opposed to high-density, outbreak populations.

Discussion

Related Prophylaxis Phenomena

Although the DDP hypothesis is framed around adaptive responses to population density, the same rationale can be applied to the adaptive allocation of resources to parasite defense in relation to any cue that reliably predicts risk of parasitic infection. For example, recently there has been interest in examining seasonal variation in immune defense (e.g. Nelson 2004). Møller et al. (2003) have argued that since many parasites time their reproduction to coincide with that of their hosts, there will be strong selection on hosts to exhibit an annual peak in their ability to mount an immune response during the breeding season. As predicted, they found that in a sample of temperate bird species, between the breeding and non-breeding seasons, spleen mass decreased by 18 percent (the spleen is the main organ for B-cell differentiation and proliferation) and T-cell mediated immunity dropped by 33 percent. Whether insects exhibit similar seasonal variation in immune function remains to be determined.

The risk of becoming parasitized may be most reliably predicted by previous infection, since infection may indicate that parasites are becoming more abundant. Thus, the adaptive immune system of vertebrates may be viewed as a prophylactic immune defense mechanism. Although acquired immunity does not exist in invertebrates, this logic has been applied to the study of the insect immune system by Moret and Siva-Jothy (2003). When an

insect is subjected to immune challenge, it produces an immune response that appears to persist for longer than is strictly necessary to clear the infection. Moret and Siva-Jothy argue that this is because these long-lasting immune responses provide increased resistance to later infections. They tested this idea by experimentally mimicking a primary immune insult (pre-challenge) in larvae of the mealworm beetle, *Tenebrio molitor*, with lipopolysaccharides (LPS; see Table 1) prior to exposure to spores of the entomopathogenic fungus, *Metarhizium anisopliae*. They found that these pre-challenged larvae produced a long-lasting antimicrobial response, which provided a survival benefit when the larvae were subsequently exposed to fungal infection. This result suggests that the long-lasting immune response of insects protects them from secondary challenges, and hence may serve a prophylactic function.

Although the DDP hypothesis refers to adaptive prophylactic changes in pathogen resistance in response to density-dependent changes in the risk of being parasitized, similar logic may also apply to anti-predator defenses (D. Whitman, pers. comm.). Thus, crypsis and aposematism may be viewed as adaptive phenotypic responses to density-dependent changes in the risk of predation. Extrapolating further, we may also observe parallels to the seasonal prophylactic responses, discussed above, in terms of adaptive responses to the seasonal risks of predation. For example, the spring brood of the caterpillar *Nemoria arizona* selects, feeds on, and resembles oak catkins, whereas the summer brood selects and feeds on oak leaves, and resembles oak leaf petioles. Thus, these divergent seasonal forms may be viewed as seasonal 'prophylactic' responses to predation risk (Greene 1989). Many age-, stage- or size-related polyphenisms (e.g., Reiskind 1970) may also be viewed as pre-emptive measures to reduce predictable changes in predation risk.

Group Living and Prophylactic Immune Defense

The DDP hypothesis concerns the allocation of resources to parasite resistance mechanisms in relation to the density-dependent increase in infection risk. It is tempting to extrapolate this hypothesis to make predictions regarding the relationship between group living and investment in parasite defense. A naïve prediction might be that, across species, we would expect group-living insects to invest more in prophylactic disease resistance than solitary-living insects. This is because group-living insects will typically experience higher local densities than solitary-living ones and hence, presumably, higher *per capita* infection risk. It has long been assumed that increased parasitism is a cost of group-living. However, the evidence for

it is equivocal at best (Freeland 1979; Davies et al. 1991; Coté and Poulin 1995). Moreover, a recent experimental comparative study indicated that in larval Lepidoptera, at least, solitary-feeding species appear to invest *more* in immune defense than do closely-related gregariously-feeding species reared under similar conditions (Wilson et al. 2003).

To examine this counter-intuitive result further, Wilson et al. (2003) developed a dynamic, susceptible/infected spatially-explicit model in which different degrees of host-clustering were generated to simulate the effect of group-living on infection risk. Using this model, Wilson and colleagues showed that, for a significant area of parameter space, host clustering could reduce the *per capita* infection risk. Thus, it appears that aggregating in clusters might actually reduce the probability of becoming infected by parasites, rather than increase it. The reason for this is as follows: if parasite transmission requires close proximity between infectious and susceptible hosts, then any process that increases the distance between individuals will lead to reduced parasite transmission. By increasing the *variance* in nearest-neighbor distance, host clustering increases the probability that the parasite will fail to breach the gap between the host it is infecting and the nearest susceptible hosts. Thus, the model indicates that part of the advantage of group-living in these scenarios is attributable to the fact that any disease epidemics will tend to fade out faster within populations of group-living animals than within populations of solitary ones (Wilson et al. 2003, see also Watve and Jog 1997). However, the costs and benefits of group living will depend critically on both the mode of parasite transmission and on the spatial structure of the host population (Wilson et al. 2003, see also Pie et al. 2003).

Melanism and Immunity in Insects

An intriguing aspect of the DDP hypothesis is its link with melanism in insects. Many phase polyphenic species are characterized by increased melanization of the cuticle, the adaptive value of which has been debated for many years. Two key hypotheses are that there could be potential thermoregulatory benefits of a dark cuticle (Johnson et al. 1985; Goulson 1994; Gunn 1998) or that the conspicuous black cuticle could play a role in aposematic signalling (Iwao 1968; Wilson 2000). However, the relationship between cuticular melanization, pathogen resistance and immune function (Kunimi and Yamada 1990; Reeson et al. 1998; Barnes and Siva-Jothy 2000; Wilson et al. 2001; Cotter et al. 2004a; Cotter et al. 2004b) suggests that the black cuticle common in high-density phenotypes may be a by-product of the up-regulation of phenoloxidase activity in the hemolymph and cuticle;

and of the strengthening role of melanin in protection against parasitoids and parasites that enter via the cuticle (e.g. entomopathogenic fungi).

As such, cuticular melanization could be seen as an immune parameter in its own right. But does cuticular melanization carry the costs expected of other immune traits? A recent study found little evidence of costs associated with cuticular melanization in the Egyptian cotton leafworm, *S. littoralis*, at least under laboratory conditions (Cotter 2004b). However, it may be that the costs of cuticular melanization are apparent only under conditions of resource competition, as has been shown for other parasite resistance traits (e.g., Moret and Schmid-Hempel 2000; Kraaijeveld and Godfray 1997; Fellowes et al. 1998). Alternatively, the costs may be associated with the increased conspicuousness to predators that is bound to occur with black larvae feeding on green foliage. Further studies examining the effects of crowding on parasite resistance in non-phase polyphenic species are required in order to elucidate the role of melanism in density-dependent prophylaxis.

Future Directions

Although evidence supporting the DDP hypothesis is slowly accumulating, the most convincing examples come from studies of larval Lepidoptera, and this taxonomic bias needs to be addressed. As well as broadening the taxonomic scope of the studies, the mechanisms underpinning DDP need to be examined at a much finer scale. Although there is some evidence relating to the immunological mechanisms that are regulated in response to changes in population density, these have so far been investigated at a fairly crude level, and there is a need for these to be refined to examine DDP responses at the cellular and molecular genetic level. There is also a need to determine precisely how gross changes in population density are translated into neurological and hormonal signals that trigger immunological and other resistance mechanisms.

Finally, although the maintenance costs of resistance have been well characterized in some species exhibiting genetically-regulated differences in levels of constitutive resistance (e.g., replicated lines of *D. melanogaster* selected for resistance to parasitoids; see above), few studies have attempted to measure the costs of resistance in species exhibiting DDP. In the Egyptian cotton leafworm, *S. littoralis*, cuticular melanism is associated with increased resistance to an entomogenous fungus (Wilson et al. 2001; see above). In this species, the melanic phenotype is smaller and has lower hemolymph protein levels (Cotter 2004a). Thus, there do appear to be small, but detectable, costs of maintaining high levels of investment in immune

function in this phase polyphenic species. In contrast, in the related African armyworm, *S. exempta*, the costs of resistance are less obvious. In this species, resistance to a range of entomopathogens (including nucleopolyhedrovirus and an ectoparasitoid) is positively associated with larval density and cuticular melanism, and the melanic, high-density phenotype has significantly higher levels of phenoloxidase activity than the non-melanic, low-density phenotype (Reeson et al. 1998; Wilson and Reeson 1998; Reeson et al. 2000). Contrary to expectation, when adult females are denied access to a carbohydrate source, moths raised under high-density conditions as larvae produce around 25% *more* eggs than those reared under low-density conditions (there was no phase difference in fecundity when the adult moths were allowed access to sucrose solution; Mensah and Gatehouse 1998). It is clear, therefore, that under laboratory conditions at least, high-density, melanic females do not appear to incur a fecundity cost to investing in immune function. Thus, a priority for future studies is to identify the costs associated with density-dependent prophylaxis.

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