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Costs of resistance: genetic correlations and potential trade-offs in an insect immune System

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Abstract

Theory predicts that natural selection will erode additive genetic variation in fitness-related traits. However, numerous studies have found considerable heritable variation in traits related to immune function, which should be closely linked to fitness. This could be due to trade-offs maintaining variation in these traits. We used the Egyptian cotton leafworm, *Spodoptera littoralis*, as a model system to examine the quantitative genetics of insect immune function. We estimated the heritabilities of several different measures of innate immunity and the genetic correlations between these immune traits and a number of life history traits. Our results provide the first evidence for a potential genetic trade-off within the insect immune system, with antibacterial activity (lysozyme-like) exhibiting a significant negative genetic correlation with haemocyte density, which itself is positively genetically correlated with both haemolymph phenoloxidase activity and cuticular melanization. We speculate on a potential trade-off between defence against parasites and predators, mediated by larval colour, and its role in maintaining genetic variation in traits under natural selection.

Introduction

By definition, parasitic infection is costly to the host. Parasites can reduce host fecundity and survival; particularly virulent parasites can kill their hosts rapidly. If this occurs in prereproductive individuals, the direct fitness of the individual is reduced to zero. It is clear, therefore, that the ability to mount an efficient and effective immune response should be closely related to fitness. It is widely assumed that natural selection will erode additive genetic variation in traits closely associated with fitness, as favourable alleles are driven to fixation (Stearns, 1992). Thus, fitness-related traits, such as life history characters and nonspecific disease resistance traits, are expected to have lower heritabilities than morphometric, and other, traits that are only weakly related to fitness. Although there is some support for this idea (Mousseau & Roff, 1987; Kruuk *et al.*, 2000; Merila & Sheldon, 2000), many studies have shown that disease resistance traits often have high heritabilities and high levels of additive genetic variation (e.g. Fellowes *et al.*, 1998; Huang *et al.*, 1999; Coltman *et al.*, 2001).

Immune function has generally been quantified in two ways. The most commonly employed method, especially in studies of invertebrates (e.g. Fuxa & Richter, 1989; Boots & Begon, 1993; Fellowes *et al.*, 1998; Huang *et al.*, 1999), is to quantify the ability of an individual to resist infection by a specific parasite. Heritability estimates obtained in this way are moderate, ranging from $h^2 = 0.24$ (Fellowes *et al.*, 1998) to 0.31 (Huang *et al.*, 1999). This is not surprising as resistance to specific parasites or pathogens is often controlled by epistatic genotype–genotype interactions, which should be under frequency-dependent selection; a mechanism which can maintain variation in fitness-related traits (Hamilton & Zuk, 1982). Whilst this method can tell us a great deal about genetic variation in parasite resistance, there are several weaknesses with this approach. For example, not only are the results necessarily specific to the particular parasite being studied, but also the efficacy of the immune system is being measured only indirectly; behavioural and other nonimmune factors may therefore confound the results.

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An alternative method to quantify immune function is to assay directly components of an individual's immune system. Generally, this will give some indication of an organism's nonspecific immune response, rather than the ability to resist a specific parasite (Norris & Evans, 2000). Although this method is more commonly used in vertebrate studies, many invertebrate species provide excellent model systems with which to examine the rules by which individuals allocate resources to immune function. This is because the invertebrate immune system is significantly simpler than that of vertebrates in that there is no acquired immunity; insects do not possess lymphocytes or immunoglobulins (Gillespie *et al*., 1997). However, the insect immune system does share many fundamental characteristics with the innate immune system of vertebrates, with many of the basic factors showing remarkable homology across species (Vilmos & Kurucz, 1998).

The few studies that have examined the quantitative genetics of immune defence in invertebrates have found significant levels of additive genetic variation in traits such as antibacterial activity, phenoloxidase (PO) activity, encapsulation ability, haemocyte density and haemocyte phagocytic ability (Kurtz & Sauer, 1999; Ryder & Siva-Jothy, 2001; Cotter & Wilson, 2002). The heritabilities of these traits ranged from moderate, $h^2 = 0.20 \pm 0.12$ for the haemocyte load of the house cricket, *Acheta domesticus* (Ryder & Siva-Jothy, 2001) to very high, $h^2 = 0.83 \pm 0.28$ for the haemocyte phagocytic activity of the scorpionfly, *Panorpa vulgaris* (Kurtz & Sauer, 1999). Therefore, significant levels of additive genetic variation can be maintained, not only in traits conferring resistance to specific parasites, but also in general components of the immune system that are mobilized in response to infection by a range of parasites.

Antagonistic pleiotropy occurs when a gene has a positive effect on one trait but a negative effect on another. This is the genetic basis of trade-offs and is the mechanism most often invoked to explain the maintenance of additive genetic variation in these systems. Only by demonstrating that there is a negative genetic correlation between traits can trade-offs be identified; phenotypic correlations are not sufficient.

A number of studies have examined correlated responses to selection for resistance to specific parasites and have identified potential trade-offs with life history traits such as development rate, competitive ability, pupal weight, adult lifespan, testes size, fecundity and egg viability (Fuxa & Richter, 1989; Boots & Begon, 1993; Groeters *et al*., 1994; Kraaijeveld & Godfray, 1997; Webster & Woolhouse, 1999; Hosken, 2001). Thus, a number of studies have demonstrated potential genetic trade-offs between parasite resistance and life history traits. Therefore, additive genetic variation in immune parameters could be maintained because high levels can be bought only at the expense of other important functions or traits. Immune function, or immunocompetence, is often thought of as a composite trait that is improved with the input of increased resources (Owens & Wilson, 1999). However, it is also conceivable that there are trade-offs within the immune system, with high levels of one component resulting in low levels of another. There is some evidence for this from vertebrate studies; particularly that upregulation of the humoral response may be at the expense of cellular responses (Gross *et al*., 1980; Siegel & Gross, 1980; Grencis, 1997; Gehad *et al*., 1999; Ibanez *et al*., 1999; Johnsen & Zuk, 1999; Gill *et al*., 2000). For example, Seigel & Gross (1980) selected chickens for high (HA) and low (LA) antibody production after inoculation with sheep red blood cells. A subsequent study found that HA birds showed higher resistance to three pathogens and feather mites, but lower resistance to two bacterial pathogens than LA birds (Gross *et al*., 1980). As bacterial infections are generally controlled by heterophils and other phagocytosing cells (Roitt *et al*., 1998), these results suggest that selection for enhancement of a component of the acquired immune system (antibody production) could lead to a reduction in the efficacy of a component of the innate immune system (phagocytes). However, few previous studies on insects have measured multiple immune function traits in the same individuals, and we know of no examples where genetic trade-offs within the insect immune system have been demonstrated.

In order to reveal potential genetic trade-offs associated with the insect's innate immune system, we used the Egyptian cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) as a model system and examined the quantitative genetics of innate immune function and other life history parameters. This noctuid moth is a phase-polyphenic species; a number of its life history traits, such as growth rate, pupal weight and larval activity, are phenotypically plastic with respect to population density (Hodjat, 1970). The most striking difference between low- and high-density phenotypes, however, is in the level of cuticular melanization. At low population densities, *S. littoralis* larvae tend to be pale brown/grey ('nonmelanic larvae') but when exposed to high larval densities, most develop a highly melanized cuticle ('melanic larvae'). Cuticular melanization has been linked to parasite resistance in a number of phase-polyphenic species (Kunimi & Yamada, 1990; Reeson *et al*., 1998; Barnes & Siva-Jothy, 2000; Wilson *et al*., 2001) and it is phenotypically correlated with PO activity levels in the haemolymph, midgut and cuticle (Wilson *et al*., 2001; Cotter & Wilson, 2002), an enzyme that has been implicated in resistance to a range of pathogens (Rowley *et al*., 1990; Hagen *et al*., 1994; Reeson *et al*., 1998). Furthermore, a previous study found that the heritability of haemolymph PO activity was extremely high in this species ($h^2 = 0.69 \pm 0.069$) (Cotter & Wilson, 2002). As the melanization of the cuticle occurs mostly in larvae living in high-density populations, in which the risk of pathogenesis is greatest, there must be a

fitness cost associated with its expression (Wilson & Reeson, 1998). Thus, we examined the genetic variance and covariance within and between a number of immune function traits (including cuticular melanization) and life history traits, in order to identify potential genetic trade-offs constraining immune function expression.

Materials and methods

Experimental design

A stock culture of *S. littoralis* was maintained in the laboratory under a 12L : 12D light regime. Larvae were reared at 25 °C and fed on a semi-artificial wheatgerm-based diet (Cotter, 2002). The culture was set up from larvae collected from two field sites, *c.* 30 km apart, near Alexandria, Egypt in 1998. Each generation, eggs were collected from at least 100 adults to reduce inbreeding and larvae from the same collection sites were added each year to maintain genetic variation. The insects were reared in a clean environment and the incidence of disease in the colony was very low.

A full-sib/half-sib design was used to determine heritabilities of immune parameters and life history traits (Lynch & Walsh, 1998). Eleven virgin males were each mated to three virgin females. However, some pairs failed to mate successfully and this resulted in 22 families in total. Of the 11 males, five successfully paired with three females each, one paired with two females and five paired with one female each. The mated females were then placed in individual containers with *ad libitum* access to 5% sucrose solution, filter paper and nappy liner on which to lay eggs. Fifty larvae from each female were removed 2 days after hatching and reared individually in 25 mL pots containing artificial diet.

Traits studied

We estimated the components of variance of four immune function traits and four life history traits (Table 1). All of the traits were measured in a low-density environment because it is only under these conditions that phenotypic variation in the degree of cuticular melanization is expressed. It is also under these low-density conditions that the costs associated with cuticular melanization, and any associated increase in immune function, are expected to be greatest.

Immune traits

Approximately 30 final instar larvae from each family were randomly selected and the colour (*cuticular melanization*) of each larva quantified (Wilson *et al.*, 2001). Three haemolymph-based immune parameters were then measured for each larva: *haemolymph PO activity, antibacterial activity* (measured using a lytic zone assay) and *haemocyte density*. All of these parameters are related to resistance to a range of parasites and pathogens and are used routinely to assay insect immune function (Eslin & Prévost, 1996; Reeson *et al.*, 1998; Kurtz *et al.*, 2000).

Haemolymph PO activity was measured using the methods of Wilson *et al.* (2001). In brief, 8 μL of haemolymph were added to 400 μL of ice-cold phosphate-buffered saline (PBS; pH 7.4) in a plastic Eppendorf tube and vortexed. Samples were frozen at −20 °C to disrupt haemocyte membranes and PO activity was assayed spectrophotometrically with L-Dopa as a substrate. This assay involved adding 100 μL of 20 mM L-Dopa to 100 μL of the buffered haemolymph and incubating triplicate samples of the mixture on a temperature-controlled VERSAmax tuneable microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA) for 20 min at 25 °C. PO activity was expressed as the absorbance read at 492 nm after this time,

Table 1 Trait mean values and heritability estimates.

Trait	Mean	*N*	Heritability	CV_A	CV_R
Life history traits					
Larval development rate (days)	15.56 ± 0.05	562	0.82 ± 0.03**	47.29	9.35
Pupal weight (g)	0.31 ± 0.002	545	0.85 ± 0.03**	16.61	6.51
Pupal development rate (days)	7.88 ± 0.04	480	0.20 ± 0.06*	5.29	10.55
Adult longevity (days)	11.21 ± 0.23	458	0.22 ± 0.07*	20.81	39.34
Immune function traits					
Cuticular melanization (OD units)	3.08 ± 0.04	608	0.36 ± 0.08**	20.99	27.97
Haemolymph PO activity ($\log_e$ OD units)	−2.18 ± 0.01	602	0.65 ± 0.11**	13.13	11.17
Antibacterial activity ($\log_e$ ng μL^{-1})	1.55 ± 0.04	588	0.63 ± 0.11**	63.02	61.05
Haemocyte density (cells per 0.2 μL)	229.91 ± 3.75	594	0.36 ± 0.08**	22.80	30.56

Mean values and heritabilities are given with their SE. *N* refers to the number of larvae sampled for each trait, there were 22 families in total. Heritabilities were estimated by partitioning the total variance into additive genetic variance and residual variance. Coefficients of additive genetic covariation (CV_A) for transformed data are meaningless (Houle, 1992), therefore CV_A and CV_R were calculated for the untransformed data.

*$P < 0.01$, **$P < 0.001$.

OD, optical density; PO, phenoloxidase.

which is during the linear phase of the reaction (Cotter, 2002).

To assess *haemocyte density*, 10 μL of haemolymph were added to 5 μL of ethylenediaminetetraacetic acid (EDTA) anticoagulant in PBS, and 5 μL of glycerol in a plastic Eppendorf tube (Cotter, 2002). The contents of the tube were gently mixed and 15 μL were pipetted onto a haemocytometer with improved Neubauer ruling. The numbers of haemocytes in five nonadjacent squares were counted on each side of the haemocytometer at ×400 magnification. The counts for each chamber were summed and averaged to give an estimate of the haemocyte density of each individual.

Antibacterial activity against the bacterium, *Micrococcus lysodeikticus* was determined using a lytic zone assay. Agar plates containing 10 mL of 1% agar with 5 mg mL^{-1} freeze-dried *M. lysodeikticus* were prepared as described in Kurtz *et al.* (2000). Holes with a diameter of 2 mm were punched in the agar and filled with 70% ethanol saturated with phenylthiourea (PTU), which inhibits melanization of the haemolymph. After the ethanol had evaporated, 1 μL of haemolymph was placed in each well, two replicates per sample. The plates were incubated at 33 °C for 24 h. After this time the plates were digitally photographed and the diameter of the clear zones calculated using IMAGE PRO PLUS software (Media Cybernetics, Silver Spring, MD, USA). Standard curves were obtained using a serial dilution of hen egg white lysozyme, from which the concentration of 'hen egg white lysozyme equivalents' in each haemolymph sample was then calculated.

Life history traits

For each individual, the following data were collected: *larval development rate* (the reciprocal of the number of days from hatching to pupation); *pupal weight* (weight in g measured 24–48 h after the start of pupal stage); *pupal development rate* (the reciprocal of the number of days from pupation to eclosion); *sex* (which was assessed for individuals that pupated successfully); and *adult longevity* (the number of days from eclosion to death).

Variance components analysis

Heritability estimates of each trait and genetic correlations between traits were estimated using a restricted estimate maximum likelihood (REML) procedure (VCE version 4, Groeneveld & Kovac, 1990; see http://w3.tzv.fal.de/~eg/vce4/vce4.html). This involved fitting an individual 'animal model', a form of mixed model where the phenotype of each individual is separated into an additive genetic component plus other random and fixed effects. First, we fitted a model with just an additive genetic effect for all 22 families such that: $\mathbf{y} = \mu + \mathbf{a} + \mathbf{e}$,where μ is the population mean, $\mathbf{y}$ is the vector of phenotypic values, $\mathbf{a}$ is the vector of additive genetic effects and $\mathbf{e}$ is the vector of residual values (Lynch & Walsh, 1998). The REML analysis estimates the additive genetic variance, V_A, from the variance–covariance matrix of $\mathbf{a}$, which is assumed to be $\mathbf{A}\ V_A$, where A is the additive genetic relationship matrix determined by the pedigree structure (Lynch & Walsh, 1998). The phenotypic variance of each trait, V_P, is thus described as $V_P = V_A + V_R$, where V_A is the additive genetic variance and V_R is the residual variance, including nonadditive sources of genetic variance such as dominance variance or epistatic effects, environmental effects and error variance. Secondly, by including maternal identity as an additional random affect, we fitted a model with both additive genetic and maternal effects, such that, $\mathbf{y} = \mu + \mathbf{a} + Z\mathbf{m} + \mathbf{e}$,where $\mathbf{m}$ is the vector of maternal effects and Z is the design matrix relating records to the appropriate maternal effect. For this model, we restricted the data to the 17 families where each male had been mated to more than one female. The second model therefore partitions the phenotypic variance as $V_P = V_A + V_M + V_R$, where V_M is the variance attributable to maternal effects. The animal model can readily be extended to multivariate analyses in VCE, so that all traits could be analysed simultaneously and their genetic correlations estimated.

We determined whether measurements made on the two sexes should be treated as single or separate traits by estimating the additive genetic variances and additive genetic covariances between the sexes. Because all traits had approximately equal additive genetic variances in the two sexes (paired t-test: $t = -1.46$, d.f. $= 6$, $P = 0.196$) and the genetic correlation across the sexes did not differ significantly from one (t-test: $t = -0.78$, d.f. $= 7$, $P = 0.230$) (Lynch & Walsh, 1998), final genetic parameters for the suite of traits measured in this study were estimated in a single multiple trait VCE analysis, using both sexes. The effects of larval 'condition' were tested in these models by including larval weight and haemolymph protein levels in the models. However, the heritability estimates and genetic correlations were similar regardless of whether or not these covariates were included in the analysis (Cotter, 2002). Therefore, these condition measures are excluded from the analyses presented here.

The heritability of each trait was calculated as the ratio of additive genetic variance to phenotypic variance: $h^2 = V_A/V_P$, and maternal effects were calculated as the ratio of maternal effects variance to phenotypic variance: $m^2 = V_M/V_P$. Genetic correlations between each pair of traits, r_A, were estimated from the genetic covariance estimate between the two traits Cov [x, y], and the estimate of additive genetic variance for each trait V_{Ax} and V_{Ay} where $r_A = \text{Cov}\ [x, y]/[(V_{Ax})(V_{Ay})]^{0.5}$. The VCE programme returns SE for all estimates, and these were used to determine the significance of parameter estimates using t-tests. As the REML procedure assumes that the data are normally distributed, haemolymph PO activity

and antibacterial activity were log-transformed prior to analysis to conform to this assumption. After transformation, all of the traits were normally distributed except for cuticular melanization; the results for this trait should therefore be viewed with some caution (though VCE is fairly robust to deviations from normality; Kruuk *et al.*, 2000).

To account for the problem of multiple testing, we used the method of Benjamini & Hochberg (1995) to control the false discovery rate, i.e. the expected proportion of false rejections within a class of rejected null hypotheses. Briefly, the $p_{(i)}$-values from the m tests are ordered from smallest to largest, $p_{(1)}$–$p_{(m)}$. If k is the largest value of i for which $pi \leq i/m\alpha$, then all the null hypotheses from 1–k are rejected. This technique is less conservative than the Bonferroni correction and so gives greater power to detect significant genetic correlations.

Results

Heritabilities

All of the measured traits exhibited significant additive genetic variation, with heritability estimates in the range $h^2 = 0.22$–0.85 (Table 1). There was no obvious difference between the heritability estimates obtained for life history and immune traits (mean $h^2_{\text{Lifehistory}} = 0.53 \pm 0.18$ SE; mean $h^2_{\text{Immunity}} = 0.48 \pm 0.07$ SE).

Genetic correlations between life history traits

There were significant positive and negative genetic correlations between many of the life history traits (Table 2). Larval development rate was positively genetically correlated with pupal weight ($r_A = 0.24 \pm 0.05$, $t = 4.80$, d.f. = 543, $P < 0.001$) and pupal development rate ($r_A = 0.20 \pm 0.08$, $t = 2.48$, d.f. = 478, $P = 0.013$). Pupal weight was positively genetically correlated with adult longevity ($r_A = 0.41 \pm 0.10$, $t = 4.12$, d.f. = 456, $P < 0.001$), and pupal development rate was negatively genetically correlated with both pupal weight ($r_A = -0.34 \pm 0.09$, $t = -3.85$, d.f. = 478, $P < 0.001$) and adult longevity ($r_A = -0.49 \pm 0.11$, $t = -4.33$, d.f. = 456, $P < 0.001$).

Genetic correlations between immune traits

There were significant positive and negative genetic correlations between the immune traits (Table 2). There were positive genetic correlations between haemocyte density and both the degree of cuticular melanization ($r_A = 0.55 \pm 0.08$, $t = 6.66$, d.f. = 592, $P < 0.001$) and haemolymph PO activity ($r_A = 0.21 \pm 0.01$, $t = 2.10$, d.f. = 592, $P < 0.05$). However, the genetic correlation between haemocyte density and antibacterial activity was significant and negative ($r_A = -0.23 \pm 0.10$, $t = 2.29$, d.f. = 586, $P < 0.05$), suggesting a possible genetic trade-off.

Genetic correlations between life history and immune traits

Each of the immune traits exhibited significant genetic correlations with one or more of the life history traits (Table 2). Cuticular melanization was positively genetically correlated with larval development rate ($r_A = 0.18 \pm 0.06$, $t = 3.27$, d.f. = 560, $P < 0.001$), but negatively genetically correlated with pupal development rate ($r_A = -0.49 \pm 0.10$, $t = -4.93$, d.f. = 478, $P = 0.001$). Haemolymph PO activity was positively genetically correlated with pupal weight ($r_A = 0.41 \pm 0.06$, $t = 6.83$, d.f. = 543, $P < 0.001$) and adult longevity ($r_A = 0.56 \pm 0.10$, $t = 5.43$, d.f. = 456, $P < 0.001$), but negatively genetically correlated with pupal development rate ($r_A = -0.38 \pm 0.11$, $t = -3.66$, d.f. = 478, $P < 0.001$). Antibacterial activity was positively genetically correlated with pupal development rate ($r_A = 0.35 \pm 0.09$, $t = 3.69$, d.f. = 478, $P < 0.001$), but negatively genetically correlated with larval development rate ($r_A = -0.29 \pm 0.08$, $t = -3.82$, d.f. = 560, $P < 0.001$). Finally, haemocyte

Table 2 Genetic and phenotypic correlations between traits.

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

Values above the diagonal show phenotypic correlations (Pearson's) between traits, values below show genetic correlations as estimated by VCE. Significance levels were determined with *t*-tests: ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All correlations remain significant after controlling the false discovery rate (Benjamini & Hochberg, 1995).
PO, phenoloxidase.

density was negatively genetically correlated with pupal weight ($r_A = -0.17 \pm 0.07$, $t = 2.60$, d.f. $= 543$, $P < 0.05$) < 0.05) and pupal development rate ($r_A = -0.66 \pm 0.09$, $t = -7.42$, d.f. $= 478$, $P < 0.001$).

Maternal effects

The significance of the mothers' identity was tested using a reduced data set of 17 families, in which each male was mated to more than one female, in order to examine potential maternal effects that may have been missed in the full, unbalanced data set, and their effect on the heritabilities and genetic correlations. Larval development rate was found to have a significant maternal effect ($m^2 = 0.43 \pm 0.09$, $t_{424} = 4.88$, $P < 0.001$); the effect for pupal weight was marginally nonsignificant ($m^2 = 0.24 \pm 0.12$, $t_{410} = 1.95$, $P = 0.051$); maternal effects were not significant for any of the other traits ($P > 0.10$). Fitting maternal effects for LDR and PW reduced their heritability estimates from 0.82 ± 0.03 to 0.42 ± 0.15 (LDR) and from 0.85 ± 0.03 to 0.49 ± 0.31 (PW), suggesting that initial estimates of their heritability were inflated by maternal effects covariance. Including maternal effects for these traits in the multivariate model of all traits did not result in a qualitative change in the overall pattern of genetic correlations although many of the SE were larger due to the reduced sample size. Despite this, the key correlations remained significant; cuticular melanization was positively genetically correlated with LDR ($r_A = 0.72 \pm 0.21$, $t_{424} = 3.44$, $P < 0.001$) and haemolymph PO activity was positively genetically correlated with PW ($r_A = 0.57 \pm 0.21$, $t_{410} = 2.75$, $P = 0.006$). Furthermore, the correlations between the immune traits were strengthened; cuticular melanization was positively genetically correlated with haemolymph PO activity ($r_A = 0.32 \pm 0.08$, $t_{456} = 3.99$, $P < 0.001$) and haemocyte density ($r_A = 0.77 \pm 0.10$, $t_{450} = 7.64$, $P < 0.001$), whilst antibacterial activity was negatively genetically correlated with both haemocyte density ($r_A = -0.30 \pm 0.09$, $t_{446} = -3.16$, $P = 0.002$) and cuticular melanization ($r_A = -0.41 \pm 0.10$, $t_{446} = -4.08$, $P < 0.001$), reinforcing our earlier conclusion of a possible genetic trade-off within the immune system.

Discussion

Maintenance of additive genetic variation

Despite the fact that many of the measured traits should be closely related to fitness, all of the heritability estimates were significantly different from zero and generally large (Table 1). There was evidence for inflation of these estimates by maternal effects for just two of the life history traits; there was no effect of maternal identity on any of the immune traits. An earlier study also found no evidence for maternal effects on haemolymph PO activity in this species (Cotter & Wilson, 2002). Two previous studies have suggested that lysozyme activity in the scorpionfly (Kurtz & Sauer, 1999) and haemocyte load in the house cricket (Ryder & Siva-Jothy, 2001) are potentially influenced by maternal effects. However, in both of these studies the offspring from full-sib families were reared in the same containers and so the heritability estimates may have been inflated by a common environment effect rather than an effect of maternal phenotype. Here, individual rearing of larvae will have minimized the potential for any such common environment effects.

Significant levels of additive genetic variation are clearly being maintained in both life history and immune traits, despite the fact that the immune traits, in particular, should be subject to strong selection pressure. This study examined evidence for trade-offs that could be maintaining this variation, namely trade-offs within the immune system, and trade-offs between immune function and life history traits.

Trade-offs within the immune system

Most studies that have attempted to examine the genetic basis for disease resistance, particularly in invertebrates, have tended to concentrate on a single measure of immune function. As a consequence, genetic correlations between different components of the immune system have rarely been examined and, as far as we are aware, no previous studies have identified any negative genetic correlations (and hence potential trade-offs) within the invertebrate immune system.

The present study has revealed a potential genetic trade-off within the immune system as haemocyte density is positively genetically correlated with cuticular melanization and PO activity but negatively genetically correlated with antibacterial (lysozyme-like) activity. Several vertebrate studies have found evidence for trade-offs between the humoral and cell-mediated arms of the immune system (Gross *et al*., 1980; Siegel & Gross, 1980; Grencis, 1997; Gehad *et al*., 1999; Ibanez *et al*., 1999; Johnsen & Zuk, 1999; Gill *et al*., 2000) but this is perhaps less surprising than the possibility of a trade-off between general components of the innate immune system. Three recent studies examining immune function in insects have identified a possible trade-off between PO activity and antibacterial activity based on negative *phenotypic* correlations between the two traits (Moret & Schmid-Hempel, 2001; Rantala & Kortet, 2003; Moret & Siva-Jothy, 2003) suggesting that this potential trade-off may occur across several insect taxa.

Melanism and DDP

The potential trade-off within the immune system appears to be linked to the melanic and nonmelanic phases associated with density-dependent phase-polyphenism (Wilson *et al*., 2001). The density-dependent

prophylaxis (DDP) hypothesis predicts that larvae living in crowded conditions, i.e. melanic larvae, will invest more resources in immune function as a result of the increased risk of pathogenesis at high densities (Wilson & Reeson, 1998; Wilson *et al.*, 2002). However, a potential trade-off within the immune system would mean that larvae could not simultaneously increase investment in all components of the immune system in response to high population densities. Instead, it appears that those (melanic) larvae predicted to do well under crowded conditions, when the infection risk is high, invest in cuticular melanization, haemocyte production and PO activity, at the expense of antibacterial (lysozyme-like) activity. In fact, a previous study using this species found that nonmelanic larvae reared under solitary conditions had high antibacterial but low PO activity, whilst melanic larvae reared under crowded conditions had high PO activity but low antibacterial activity, providing further evidence that this negative genetic correlation represents a real trade-off within the immune system (Cotter *et al.*, 2003).

The potential trade-off within the immune system may be hormonally controlled. Hormones are excellent candidates for the mediation of life history trade-offs as not only can a single hormone have different effects on different targets, but also their effects may vary depending on the life-stage of the organism (Finch & Rose, 1995). There have been few studies investigating the hormonal regulation of immune function in insects. However, a recent study found that juvenile hormone (JH) can inhibit PO activity in *Tenebrio molitor* (Rolff & Siva-Jothy, 2002). It is also well documented that JH application can cause locusts to switch from the conspicuous high-density form to the green solitary form, and that the pale, solitary forms of many phase-polyphenic Lepidoptera have higher JH titres than their crowded, dark counterparts (Yagi & Kuramochi, 1976; Ikemoto, 1983; Fescemyer & Hammond, 1988; Pener, 1991). It is therefore possible that the trade-off in the immune system is hormonally regulated with the high JH titres of nonmelanic larvae inhibiting haemocyte production, PO activity and cuticular melanization whilst promoting antibacterial activity. Further studies are clearly required to clarify the role of hormones in regulating insect immunity.

Trade-offs between immunity and other life history traits

Implicit in the DDP hypothesis is the assumption that immune function is costly (Wilson & Reeson, 1998). We would therefore expect that in conditions where the risk of pathogenesis was low, dark, melanic larvae would be out-competed by pale, nonmelanic larvae. Based on the significant genetic correlations between traits, we can consider that melanic larvae represent a phenotype characterized by investment in cuticular melanization, haemocyte density and PO at the expense of antibacterial activity. Contrary to expectation, the only costs associated with the dark phase in *S. littoralis* appear to be lower antibacterial activity and a slower pupal development rate. As the larval development period is longer than the pupal development period (Table 1), the amount of time spent as a juvenile (hatching to eclosion) is primarily determined by the larval development rate. It seems unlikely, therefore, that a decreased pupal development rate inflicts a large enough cost to counter the benefits of a fast larval development rate. Thus, there is very little evidence for a life history cost associated with melanism (and disease resistance) in this system.

Trade-off between immunity and predation avoidance

One possibility to explain the apparent lack of a cost to being melanic is that the costs are revealed only under particular field conditions. For example, it is possible that the cost of being melanic is increased conspicuousness to predators. At low population densities, the increased conspicuousness of melanic larvae may outweigh any potential benefits in terms of increased disease resistance. Because disease transmission is usually positively density-dependent, as larval density increases, the relative benefits of being melanic and resistant will outweigh the *per capita* predation risk, which is likely to be reduced at high densities as a result of 'dilution effects'. Thus, at low larval densities selection should favour crypsis over disease resistance, whereas at high densities disease resistance should be more important (Sword, 2000; Wilson, 2000). Further experiments are required to test whether the trade-off between defence against parasites and predators occurs in this species.

Conclusions

To our knowledge, this study is the first to identify a potential genetic trade-off within the insect immune system. An apparent lack of costs associated with the dark phase of larvae leads us to speculate that there could be a trade-off between defence against pathogens (investment in immunity) and predators (crypsis). Future research should examine the importance of environmental effects on these trade-offs, particularly how the relationships change under conditions of high competition, as experienced in high-density, outbreak populations.

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