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Effects of pathogen exposure on life-history variation in the gypsy moth (*Lymantria dispar*)

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sexual size dimorphism;
trade-offs.

Abstract

Investment in host defences against pathogens may lead to trade-offs with host fecundity. When such trade-offs arise from genetic correlations, rates of phenotypic change by natural selection may be affected. However, genetic correlations between host survival and fecundity are rarely quantified. To understand trade-offs between immune responses to baculovirus exposure and fecundity in the gypsy moth (*Lymantria dispar*), we estimated genetic correlations between survival probability and traits related to fecundity, such as pupal weight. In addition, we tested whether different virus isolates have different effects on male and female pupal weight. To estimate genetic correlations, we exposed individuals of known relatedness to a single baculovirus isolate. To then evaluate the effect of virus isolate on pupal weight, we exposed a single gypsy moth strain to 16 baculovirus isolates. We found a negative genetic correlation between survival and pupal weight. In addition, virus exposure caused late-pupating females to be identical in weight to males, whereas unexposed females were 2–3 times as large as unexposed males. Finally, we found that female pupal weight is a quadratic function of host mortality across virus isolates, which is likely due to trade-offs and compensatory growth processes acting at high and low mortality levels, respectively. Overall, our results suggest that fecundity costs may strongly affect the response to selection for disease resistance. In nature, baculoviruses contribute to the regulation of gypsy moth outbreaks, as pathogens often do in forest-defoliating insects. We therefore argue that trade-offs between host life-history traits may help explain outbreak dynamics.

Introduction

The evolutionary significance of life-history trade-offs partly depends on the magnitude and sign of genetic covariances between traits (Lande, 1979; Roff, 1992; Stearns, 1992). Host–pathogen interactions provide a useful opportunity to study life-history trade-offs because pathogens affect the allocation of host resources between survival and fecundity, in addition to causing host mortality (Sheldon & Verhulst, 1996). Moreover, trade-offs between pathogen-specific immune responses and other life-history traits are widespread in nature (Bergelson & Purrington, 1996; Kraaijeveld & Godfray, 1997; Lochmiller & Deerenberg, 2000; Råberg et al., 2009). However, few studies of animal host–pathogen systems have estimated the genetic variances and covariances for traits associated with immune responses and reproduction. Our lack of knowledge about the genetic covariation between these life-history traits thus makes it difficult to know the extent to which trade-offs affect the evolution of disease resistance (Stearns, 1989).

Pathogen-driven life-history trade-offs may also be sex specific if males and females differ in how they allocate resources to fecundity and immunity (Stillwell et al., 2010). Indeed, in many animal species, including the forest Lepidopteran studied here, females increase fecundity by investing in large body sizes (Hendry & Stearns, 2004; Fairbairn et al., 2007). This high investment in growth and fecundity potentially reduces the allocation of resources to other life-history processes, such as immune defenses. Consequently, if hosts are exposed to

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their pathogens, the necessary investment in the immune response may have a strong effect on female but not male body size. Moreover, the evolution of disease resistance may be affected in complex ways if differences in resource allocation patterns between males and females are genetically based (Conner, 2012). However, little is known about the effect of immune responses on sex-specific body sizes and whether pathogen-driven life-history trade-offs play a role in determining body size differences between females and males (for the effect of other environmental variables on sexual size dimorphism, see Teder & Tammaru, 2005; Fairbairn et al., 2007; Stillwell et al., 2007; Stillwell et al., 2010).

Here, we use the gypsy moth (*Lymantria dispar*) and its baculovirus to quantify trade-offs between fecundity and disease resistance and to determine whether pathogen exposure affects the sexual size dimorphism characteristic of this insect species. To do this, we quantify survival given virus exposure (hereafter referred to as survival probability), pupal weight and the time to pupation, all of which are key traits affecting disease resistance and fecundity. Baculoviruses are common pathogens of many Lepidoptera and are typically transmitted when insect larvae consume foliage contaminated with the infectious cadavers of conspecifics (Cory & Myers, 2003). Under laboratory conditions, gypsy moths are easy to rear, and exposure to the baculovirus can be carefully controlled in terms of dosage. We therefore conducted a series of laboratory experiments to estimate the genetic correlations between life-history traits affected by pathogen exposure, and to quantify the effect of pathogen exposure on the extent of sexual size dimorphism in this species.

Our results show that disease resistance is partly determined by genetic variation and that there is a negative genetic correlation between resistance and pupal weight, consistent with a resistance–fecundity trade-off. Moreover, our data strongly suggest that the trade-off between resistance and pupal weight is due to resource allocation trade-offs rather than to size-selective effects. Our results also show that virus exposure has sex-specific effects on the measured traits, leading to strong nonlinear effects on sexual size dimorphism. Our results thus show that pathogens can play a complex but important role in determining life-history trait variation in gypsy moths. The virus also helps drive gypsy moth population cycles in nature (Dwyer & Elkinton, 1993; Myers, 2000; Moreau & Lucarotti, 2007), and so our work has implications for gypsy moth population dynamics.

Materials and methods

Study organisms

In the gypsy moth, baculovirus epizootics begin when hatchling larvae chew their way out of virus-contaminated eggs (Murray & Elkinton, 1989). Virus-killed hatchlings then release infectious occlusion bodies onto foliage, which may eventually be consumed by uninfected larvae in later larval stages known as 'instars', completing the transmission cycle. In high-density gypsy moth populations, repeated rounds of transmission can lead to cumulative infection rates that often exceed 90% (Woods et al., 1991). Epizootic intensity is typically determined by infection rates among larvae in the third and fourth instar, as these instars display high feeding rates (Woods & Elkinton, 1987). We therefore used fourth instars in our experiments. Following virus infection under laboratory conditions, larvae die within 7 to 25 days (Kennedy et al., 2014). At death, the insect's integument is digested by viral proteases and new occlusion bodies are released into the environment (Washburn et al., 1996). Surviving larvae pupate after insects grow through either five (in males) or six (in females) instars. The gypsy moth has only one generation per year, and so the virus overwinters by contaminating the egg masses laid by surviving females. This leads to the reintroduction of the virus into the larval population the following spring.

Gypsy moth collection and rearing protocol

To rear gypsy moths under pathogen-free conditions, we followed standard protocols that eliminate unwanted infections prior to virus exposure (described in Dwyer & Elkinton, 1995). All larvae were reared in groups of 30 individuals, in plastic cups containing a standard artificial gypsy moth diet (Keena & ODell, 1994). These cups were held in a growth chamber at 25 °C, using a 14 : 10 light–dark cycle. For this study, we used gypsy moths collected both from the wild and from a laboratory population known as the New Jersey Standard Strain (Keena & ODell, 1994). We will refer to these two groups as the wild-collected and the laboratory strain, respectively.

We used the wild-collected gypsy moths in an experiment to estimate quantitative genetic parameters. To do this, we raised gypsy moths for two generations and conducted specific matings that produced individuals of known relatedness (Fig. 1). The first generation consisted of 101 full-sib families collected from the wild across sites in Michigan, Wisconsin, Indiana and Illinois, in the upper midwest of the US. These individuals were reared to adulthood and used as parents to create 23 paternal half-sib families by mating 1 male with two or more unrelated females. Furthermore, because we used multiple full siblings from a given parental egg mass as progenitors, our complete mating design, across 1622 insects, also included parent–offspring, full-sib, first cousin and double first cousin relationships (Fig. 1). The two generations of gypsy moths were reared under the same laboratory conditions, following standard protocols (McManus & Doane, 1981).

The laboratory strain of gypsy moths was used to test the effect of variation in baculovirus isolate and dose

on both pupal weight and sexual size dimorphism. In contrast to the wild-collected insects, the laboratory strain insects display less variation in developmental rates and survival probability (Dwyer et al., 1997), owing to continuous breeding under laboratory conditions for over 40 generations (Keena & ODell, 1994).

Virus exposure protocol

We exposed gypsy moth larvae to the virus at the beginning of the fourth instar by feeding them 3 mm^3 cubes of artificial diet together with 3 μl of virus solution. The small number of larvae that failed to consume the entire dose within 24 h was discarded. Control insects were treated identically, except that their diet cubes received 3 μl of dH_2O. We used fourth instars that were developmentally synchronized to minimize variation in survival probability that can occur due to developmental differences between and within instars (Grove & Hoover, 2007). Using larvae in developmental synchrony also ensures that the variance in the outcome of the experiments is indistinguishable from binomial sampling error (Dwyer et al., 2005).

In the quantitative genetics experiment with wild-collected insects, each larva was fed 600 occlusion bodies, a dose that caused 82% mortality. By exposing larvae to the baculovirus, however, we may have imposed selection for reduced body weight, thereby potentially confounding resource allocation trade-offs with size-selective effects. To test for this possibility, we weighed larvae shortly before exposure and compared larval weights between (i) larvae that were exposed and survived to pupation, (ii) larvae that were exposed and died from infection and (iii) larvae that were unexposed and served as controls.

Our preliminary work also suggested that the effects of virus exposure on host traits vary across pathogen isolates (see Fig. S1). We therefore used the laboratory strain of gypsy moths to conduct an additional experiment in which we directly tested the effect of 16 virus isolates on survival and pupal weight. For each isolate, we used four doses (1000, 10 000, 30 000 or 90 000 occlusion bodies) to capture isolate-specific differences in dose–response curves (see Supplementary Information for additional details). We used sixty larvae for each combination of isolate and dose, and an additional sixty insects served as an unexposed control group. Note that we specifically used the laboratory strain of gypsy moths in this experiment because the reduced phenotypic variability that characterizes this group of insects allowed us to better discern the effects of different pathogen isolates on host traits.

We recorded individual survival, pupal weight and the time to pupation over 1.5 months after exposure, a period long enough to ensure that all larvae either died or pupated. Notice, however, that pupal weight could not be measured for infected larvae because baculovirus infection is fatal. This forced us to estimate genetic covariances between survival probability and both pupal weight and the time to pupation from measure-

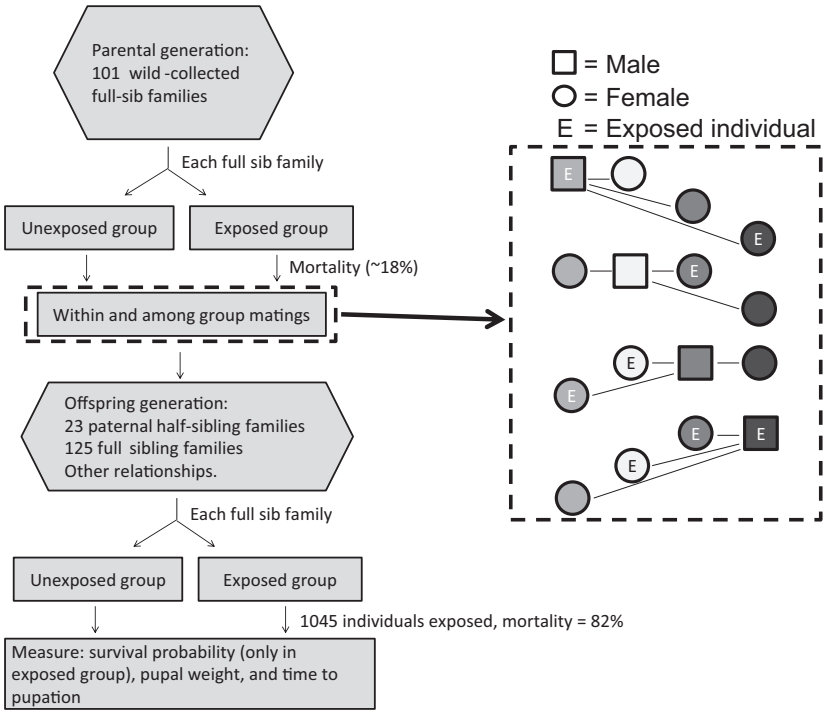

Fig. 1. Design of an experiment using wild-collected gypsy moths to measure survival probability, pupal weight and time to pupation. The left figure describes the steps used to obtain data suitable for estimating quantitative genetic parameters. The right figure shows an example of the matings conducted. In this figure, full siblings share the same shade of grey. Such matings allowed us to produce individuals sharing several genetic relationships that included paternal half-sibs and first and double first cousins. Using both exposed and unexposed individuals as parents, we were also able to test for transgenerational effects of virus exposure (Fig. 2c, d).

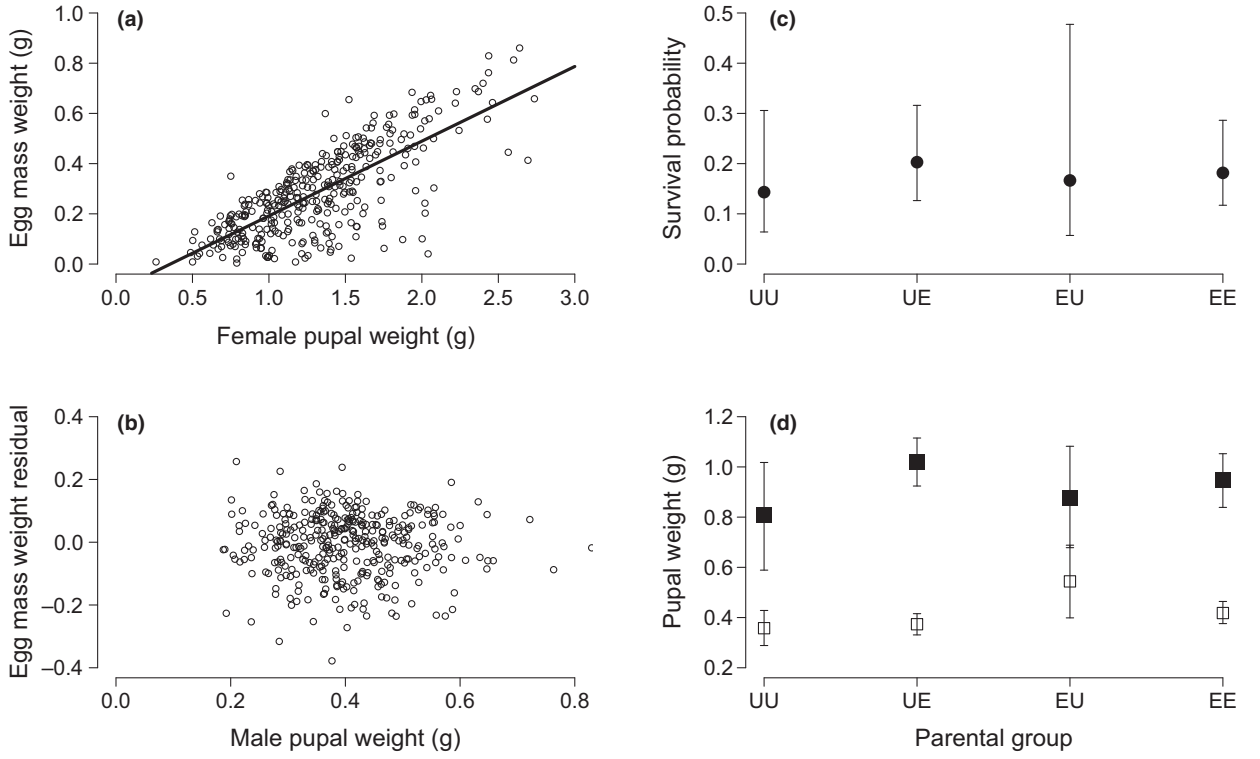

Fig. 2. Relationship between female pupal weight and the weight of the deposited egg mass, both traits measured in (g). Female pupal weight explains approximately 57 % of the variation in egg mass weight (a), based on the adjusted r^2 from a linear model. This relationship is described by $EM = -0.106+0.298PM$, where EM is the weight of the egg mass, and PM is the female pupal weight. We then used the residuals of this relationship to show that male weight is not related to egg mass weight (b). Figures c and d show no transgenerational effects of virus exposure. Offspring had parents that were either exposed (E) or unexposed (U) to virus and were categorized into UU, UE, EU and EE groups, where the first letter represents the treatment associated with the male parent. After measuring survival probability in the offspring, we used a linear mixed effects model with the parental exposure group as a fixed effect and the effects of the sire, dam and rearing cup environment as random effects. To test whether the parental exposure group contributes to explaining survival probability, we compared this model with a simpler model omitting the parental exposure group effect. As Figures c and d suggest, the parental group had no effect on offspring survival probability or pupal weight. In Figure d, the closed and open symbols are data for females and males, respectively (see further details in Fig S1).

ments made on different insects. As we explain in more detail below, existing statistical methods solve this problem by using the genetic relatedness between individuals to model the covariance between traits. Such analyses are analogous to the traditional approach of approximating the genetic correlation using the correlation of family means (Falconer & Mackay, 1996). By instead including information from all relatives in the mating design, current statistical methods have the advantage of reducing the uncertainty of parameter estimates while avoiding violations of statistical independence (Kruuk, 2004; Wilson et al., 2010).

Statistical analyses

Estimation of genetic parameters for the measured traits

To estimate the heritabilities of survival probability, pupal weight and the time to pupation, we employed mixed effects models that use the relatedness coefficients between individuals, as specified by the mating design (Lynch & Walsh, 1998). Because survival probability is measured as a binary trait (recorded as 0's and 1's), we assumed that it followed a binomial error distribution. To estimate the heritability of survival probability, we therefore used a generalized mixed effects model and a logit-link function. We included the cohort (i.e. parental or offspring generation) and virus treatment as fixed effects, and we modelled the effects of the individual and the common rearing environment as random effects. As the residual variance of the underlying logit probability cannot be observed, we fixed the residual variation at 1 (Hadfield, 2010). From this model, we estimated the heritability of survival probability as $h^2 = \left(\sigma_A^2\right)/\left(\sigma_A^2 + \sigma_{CE}^2 + 1 + \pi^2/3\right)$, where σ_A^2 and σ_{CE}^2 are the variances due to the individual (which captures additive genetic effects) and to the common rearing environment, respectively. The term

$\pi^2/3$ is the variance of a logistic distribution (Hadfield, 2010).

To estimate the heritabilities of pupal weight and the time to pupation, we fit a linear mixed effects model to the standardized values of each trait. Standardization was carried out with respect to individual sex, cohort and treatment group, by subtracting each individual measurement from the mean and dividing by the group's standard deviation. The linear mixed effects model for each trait then included virus isolate treatment, individual sex and cohort as fixed effects. As in the previous model, the effects from the individual and the rearing cup were included as random effects. We then estimated heritability as the ratio of the additive genetic variance to the total phenotypic variance. Notice that we did not include a maternal effect in the estimation of heritabilities because models that omitted this variable fit the data much better for all measured traits (Table S3).

To estimate genetic correlations between the measured traits, we then employed a multivariate version of these mixed effects models, with the bivariate response variable consisting of a combination of two traits. In these bivariate models, some insects from any given family were measured for survival probability, whereas other insects from the family were measured for pupal weight and the time to pupation. The model then takes into account the fact that these insects were not statistically independent, and again uses the information provided by the relatedness among all individuals to estimate the magnitude of this dependency, which is equivalent to the genetic covariance (Kruuk, 2004).

For each bivariate model, we fit the same explanatory variables as in the univariate cases. In addition, we estimated covariances between traits due to common environmental effects, but only if the model containing this covariation was more informative than a model that omitted it (Table S4). Genetic correlations (r_g) were then estimated as $r_g = \left(\mathrm{COV}(\mathrm{Trait}_1, \mathrm{Trait}_2)\right) / \left(\sigma_{\mathrm{Trait}_1}\sigma_{\mathrm{Trait}_2}\right)$, where $\mathrm{COV}(\mathrm{Trait}_1,\mathrm{Trait}_2)$ is the covariance between any two of the three traits, and $\sigma_{\mathrm{Trait}_1}$ and $\sigma_{\mathrm{Trait}_2}$ are the respective genetic standard deviations (Falconer & Mackay, 1996).

The significance of the (co)variance components was assessed by examining the 95% credible intervals obtained from the posterior distribution. In these analyses, we used the package MCMCglmm (Hadfield, 2010) available in R (R Core Team, 2012) for model fitting and estimation. In all of our analyses, we used Markov chain Monte Carlo (MCMC) with uninformative priors (Hadfield 2010). We discarded the first 15 000 steps of our MCMC chains, out of a total of 2×10^6 iterations, and we used the results from only 1 in every 1000 steps, which assured a negligible (< 0.1) autocorrelation between posterior samples.

Effects of virus exposure on male and female pupal weight and time to pupation

To test for effects of pathogen exposure on the relationship between pupal weight and the time to pupation, we used a linear mixed effects model on data from the wild-collected gypsy moths. In this model, we used pupal weight as the response variable and the time to pupation, the virus treatment and their interaction as fixed effects. The effects of sire, dam and rearing cup were fit as random effects. A significant interaction between virus treatment and the time to pupation would suggest that the relationship between pupal size and the time to pupation changes due to virus exposure. To evaluate whether this interaction better explained the data, we used the deviance information criterion (DIC) and uncertainty estimates of the parameters to compare models that either included or omitted the interaction. This analysis was conducted separately for males and females.

To then test whether pathogen exposure affects pupal weight differences between males and females, we first calculated a sexual size dimorphism index, which we used as our response variable. This index is $\mathrm{SDI} = (\bar{x}_f/\bar{x}_m) - 1$, where $\bar{x}_f$ and $\bar{x}_m$ are the mean pupal weights for females and males, respectively. The SDI index allowed us to quantify proportional differences in size, with positive values indicating how much bigger females are compared to males (e.g. SDI = 0.5 means that females are 50% larger than males; Fairbairn et al., 2007). We then used linear models to test for effects of virus exposure on sexual size dimorphism.

In explaining this calculation, it is important that we emphasize two key features of gypsy moth biology. First, males begin pupating 7–9 days before females. Second, in both sexes, pupal weight declines dramatically with increasing time to pupation. Comparing pupal weights of males and females that pupated on the same day would thus lead to incorrect inferences about the effects of virus exposure and pupation time on sexual size dimorphism. We therefore corrected for differences in pupation time in the following way. First, we calculated the mean time to pupation for males and females separately. Second, from the time to pupation for each male, we subtracted the mean time to pupation in males, and similarly for females. This approach produced a sex-specific, mean-corrected development time for each individual.

To account for error in the size dimorphism index, we used bootstrapping to generate a distribution of the size dimorphism statistic. We did this by randomly resampling the data 10^4 times, calculating the size dimorphism index at each sample. Then, to each of the 10^4 data samples, we fit a linear model in which the size dimorphism was a function of the time to pupation. Finally, we used the resulting 95th percentiles of the linear model coefficients to test for an effect of virus exposure on the relationship between size dimorphism and the time to pupation.

Effects of pathogen variability on male and female pupal weight

We used data from the laboratory strain of gypsy moths to test for effects of virus isolate variability and dose on male and female pupal weight. In these analyses, we used the fraction of individuals infected to summarize the effects of virus isolate and dose on survival. We then fit models in which either female or male pupal weight was a function of the fraction of infected individuals. We also fit a linear model with size dimorphism as the response variable and the fraction infected as the independent variable. Because there was evidence of nonlinearities, we used AIC analyses (Burnham & Anderson, 2002) to choose between quadratic, linear and null models. We then bootstrapped male and female pupal weights and the dimorphism index as described above, fitting the regression model at each bootstrap step, to calculate 95th percentiles on all regression coefficients.

Results

Estimates of genetic (co)variances and genetic effects of virus exposure

Overall, we exposed 1045 wild-collected larvae to the virus and obtained 82% mortality. We also had a total of 577 control pupae distributed across all families. Our results show that female, but not male, pupal weight is correlated with the number of eggs produced (Fig. 2a, b). Female pupal weight is therefore a good proxy for female fecundity. Additional summary statistics for the measured traits are shown in Table 1. Also, note that we did not find evidence for population differences in survival probability (Fleming-Davies, A., Dukic, V., Andreasen, V. & Dwyer, G., In Prep) or for transgenerational effects of virus exposure (Fig. 2 c, d) and so we did not include such effects in our statistical analyses.

Our quantitative genetic results show that pupal weight, time to pupation and survival probability are significantly heritable, with 95% credible intervals on the narrow-sense heritability values that exclude 0 (Table 2A). We also found significant pairwise genetic correlations (Table 2B). In particular, survival probability is positively correlated with the time to pupation, whereas pupal weight is negatively correlated with both survival probability and the time to pupation. These results suggest genetic trade-offs between disease resistance, as measured by survival probability, and traits that affect female fecundity.

As mentioned above, an alternative explanation for the negative genetic correlation between survival and pupal weight is that our experiment imposed selection against rapidly growing larvae, thus favoring smaller pupal weights. As Fig. 3 shows, however, at the time of exposure, larvae that were exposed and survived to pupation had a higher body weight on average than larvae that were exposed and ultimately died from virus infection. We also found that larval weight at the time of exposure was weakly but positively correlated with pupal weight (linear model for females, $F_{1,292} = 16.04$, P-value < 0.001, adjusted $r^2 = 0.05$; linear model for males, $F_{1,305} = 13.84$, P-value < 0.001, adjusted $r^2 = 0.04$), and so there was no indication that large larvae produced smaller pupae. We can therefore reject the hypothesis that selection for small sizes caused the negative correlation between pupal weight and survival probability, because that hypothesis implies that smaller larvae should have higher survival.

Table 1. Summary statistics for traits from the quantitative genetics experiment

Sex	Virus treatment	N survived	Pupal weight	Time to pupation
Females	Control	287	0.91 (0.24)	50.2 (6.2)
	Exposed	88	0.97 (0.27)	47.8 (6.3)
Males	Control	290	0.35 (0.10)	41.4 (7.2)
	Exposed	104	0.39 (0.11)	39.5 (8.5)

N survived refers to the number of individuals that successfully pupated following virus exposure. Pupal weight and the timing of pupation are in g and days since hatching, respectively. Values in parentheses are 1 standard deviation from the mean. We used 1045 4th instar larvae in the exposed treatment.

Table 2. Trait heritabilities and genetic correlations for survival probability, pupal weight and the timing of pupation

(A)

Trait	Mean (SD)	h^2 (95% CI)
Survival probability	0.26 (0.44)	0.25 (0.08, 0.46)
Pupal weight males	0.36 (0.10)	0.31 (0.18, 0.45)
Pupal weight females	0.92 (0.25)	0.35 (0.21, 0.51)
Pupal weight overall	–	0.31 (0.21, 0.42)
Time to pupation males	40.2 (7.64)	0.61 (0.36, 0.87)
Time to pupation females	49.0 (6.42)	0.58 (0.39, 0.78)
Time to pupation overall	–	0.40 (0.29, 0.51)

(B)

Trait correlation	r_g (95% CI)
Survival probability - Pupal weight	**−0.68 (−0.89, −0.44)**
Survival probability - Time to pupation	**0.79 (0.60, 0.95)**
Pupal weight-Time to pupation	**−0.57 (−0.77, −0.36)**

(A) Overall and sex-specific estimates of narrow-sense heritability (h^2) with 95% credible intervals (CI) in parentheses. Trait means and standard deviation (SD) were calculated across treatments. Notice that the means for the overall pupal weight and time to pupation are 0 due to trait centring. (B) Estimates of genetic correlations (r_g) between the measured traits. Bold values refer to estimates that overlap neither 0 nor -1 (see Appendix Table S2 for complete partitioning of phenotypic variation).

Our results instead show that even though heavier larvae had higher survival, final pupal weight was negatively correlated with survival probability. Our results thus suggest that pathogen exposure causes resources that would otherwise be devoted to increased growth and large pupal sizes to instead be diverted to combating pathogen attacks.

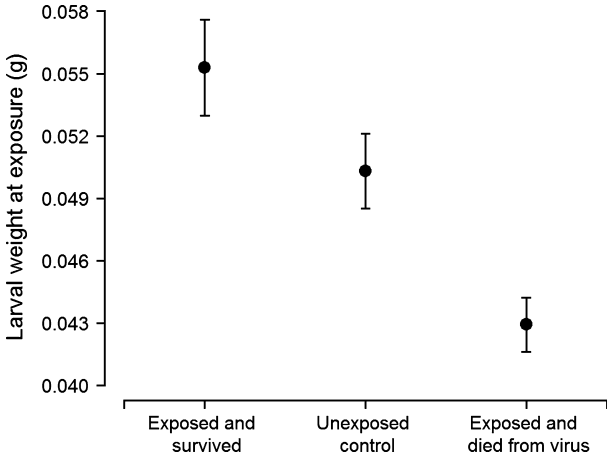

Fig. 3. Comparison of larval weights at exposure of insects that were exposed to virus and survived to pupation (exposed and survived), were unexposed and served as control (unexposed control) and died from virus infection (exposed and died from virus). Symbols are the means across groups with error bars representing the standard error of the mean × 1.96. Results from a linear model show strong differences in larval weight at exposure between the three groups (ΔAIC = 85). Similarly, a *post hoc* parametric test for multiple comparisons showed significant differences between the three groups below the threshold *P*-value of 0.05 (ANOVA, $F_{2,1366} = 45.89$, *P*-value <0.001).

Phenotypic effects of virus exposure

Our data on wild-collected insects also show that virus exposure can affect the relationship between the time and weight at pupation. In the control insects, pupal weight declined with time to pupation in females, but not in males (Fig. 4a). In the exposed insects, female pupal weight appeared to decline even more rapidly with time to pupation, although the evidence for this effect was only moderately strong (ΔDIC = 2.67 for the model omitting the interaction. Female slope in control group: −0.012, 95% CI = −0.016, −0.007; female slope in the exposed group: −0.021, 95% CI = −0.029, −0.013; Fig. 4b). In contrast, virus exposure had the opposite effect on males, in that pupal weight slightly increased with the time to pupation (male slope in control group: −0.0021, 95% CI = −0.004, −0.0005; male slope in exposed group: 0.0015, 95% CI = −0.0007, 0.004), an effect for which the evidence was stronger (model comparison ΔDIC = 4.14).

The moderate effects of virus exposure on the time and weight at pupation seen in each sex translated into strong effects on sexual size dimorphism. Specifically, for the exposed larvae, the size dimorphism index (SDI) decreased with increasing time to pupation (Fig. 4d). Contrastingly, there was no effect of time to pupation on sexual size dimorphism in the control group (Fig. 4c). We also found that a model including an interaction between virus exposure treatment and time to pupation fit the data much better than a model

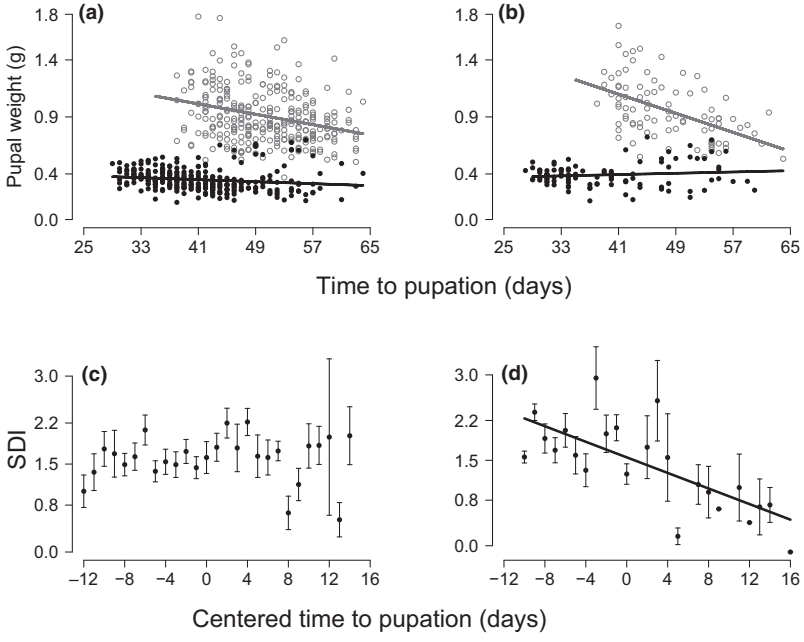

Fig. 4. Top panels: relationships between age and weight at pupation for insects in control (a) and exposed (b) treatments. Open and closed symbols refer to females and males respectively, with regression lines drawn from the model coefficients specific to each treatment. Bottom panels: relationship between sexual size dimorphism index (SDI) and the centred timing of pupation for individuals in the control (c) and exposed (d) treatments. Error bars on SDI values give the bootstrapped 95th percentiles. For SDI calculations, time to pupation is centred around male and female means, such that the earliest pupating females align with the earliest males. The regression line is drawn from the median coefficients of a bootstrapped linear model [intercept = 1.55 (1.41, 1.71), slope = −0.07 (−0.08, −0.05)].

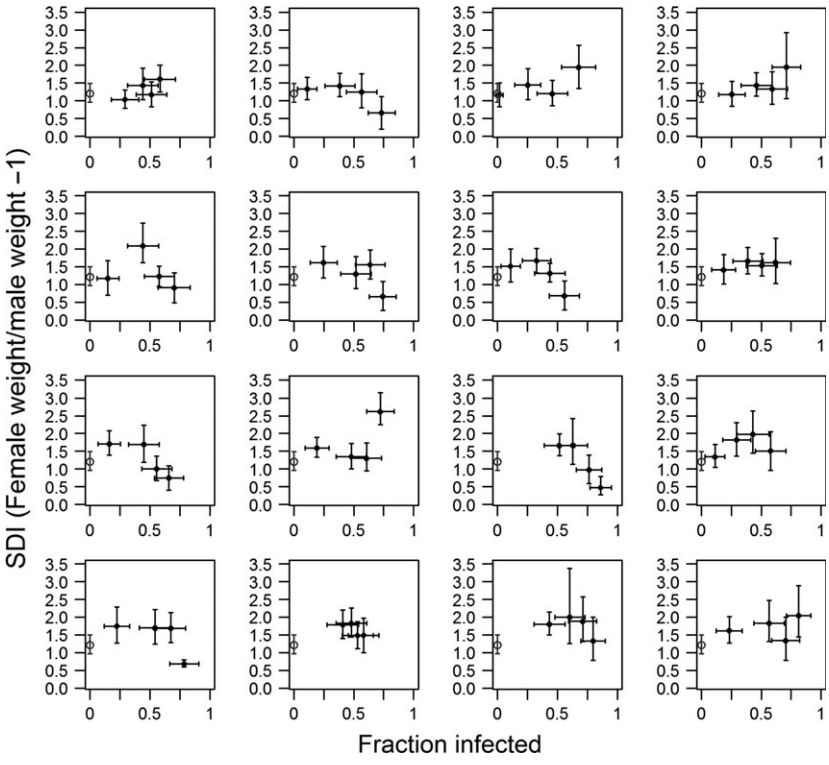

Fig. 5. Changes in host sexual size dimorphism (SDI) with exposure to each of 16 field-collected virus isolates at four doses (one plot per isolate; one point per dose). Plots are ordered by mean SDI per isolate over doses, increasing left to right and top to bottom. SDI for control (unexposed) larvae is repeated in all plots (light grey open circles). Error bars are 95% bootstrapped confidence intervals for SDI, and ±2 SE from a normal approximation to the binomial distribution describing the fraction of infected larvae.

omitting it (ΔAIC = 14, Fig. 4d). Virus exposure can thus cause late-pupating females to be very similar in weight to males through small sex-specific effects on the time and weight at pupation.

Using the laboratory strain of gypsy moths, we further show that pupal weight and sexual size dimorphism varied across both virus isolates and dosages (Fig. 5). These results also show that sexual size dimorphism is a quadratic function of the fraction of infected individuals, pooled across isolates and doses (ΔAIC = 3.35 for quadratic compared to linear model, Fig. 6). Importantly, the quadratic trend was mainly driven by pathogen effects on female pupal weight, which were similarly quadratic (ΔAIC = 3.19, Fig. 6b). Indeed, our results suggest no significant effect of viral infection on male pupal weight (Fig. 6c), as neither a linear model nor a model including a squared term fit the data better than a null model (ΔAIC = −2.00 for linear compared to null model. Note that the ΔAIC score is exactly -2 because the linear model converged on the null model).

Discussion

Our measurements of heritability and genetic correlations show that there is a strong potential for natural selection to drive changes in survival probability, pupal weight and the time to pupation. Furthermore, our results reveal trade-offs between these traits, suggesting that selection on disease resistance can have effects on traits related to fecundity and vice versa. Such trade-offs should thus be considered when evaluating the role that baculoviruses play in driving gypsy moth disease resistance in natural populations.

Previous work has also found that traits affecting insect immunity have high heritabilities (Cotter et al., 2004; Rolff et al., 2005). Regarding the associated fitness costs, however, previous studies have produced contradictory results. For example, Milks et al. (2002) found no costs associated with evolved resistance to an NPV in the cabbage looper. In contrast, both Boots & Begon (1993) and Tidbury (2012) found that evolved granulosis virus resistance negatively affected other life-history traits in the Indian meal moth. Interestingly, some studies have also found that the fitness costs of disease resistance may change depending on the quality of the rearing environment (Luong et al., 2007; Boots, 2011; Tidbury, 2012) . In contrast to previous studies, our results provide estimates of the extent to which the costs of resistance arise from genetic effects, allowing us to better understand the potential response of disease resistance to natural selection.

Our results specifically suggest that the negative genetic correlation between survival and pupal weight results from different resource allocation strategies between disease resistance and somatic growth, which ultimately affect the amount of resources available to invest in reproduction. In our experiment, larvae that

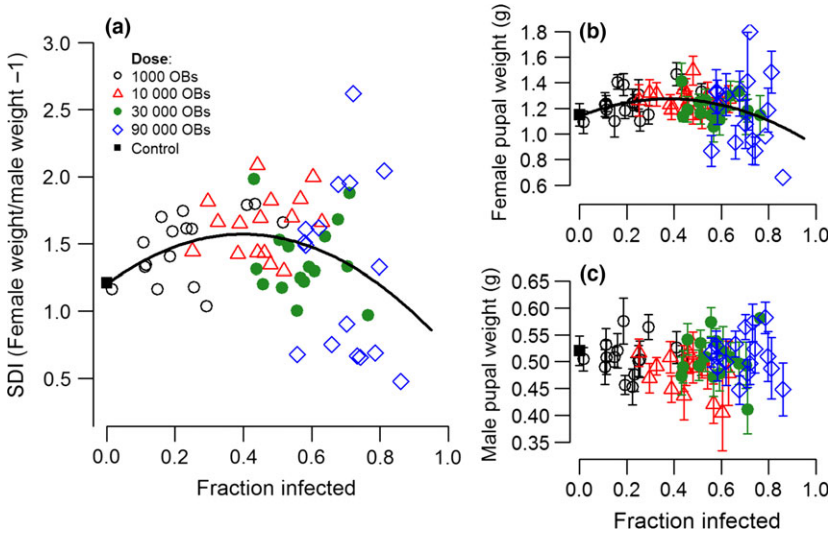

Fig. 6. Sexual size dimorphism (SDI) changes nonlinearly with virus exposure (a), primarily due to a change in female pupal weight (b). Each point in (a) is the mean SDI and fraction infected for a single virus isolate at a single dose (16 isolates total at each dose). Dose was measured as the number of infectious particles. Error in the estimated SDI and fraction infected was shown in Figure 5 and is omitted here for clarity. The line in (a) gives the fitted values from a linear regression that incorporates both a linear and a squared term for the effect of fraction infected on SDI (linear coefficient: 1.87, 95% bootstrapped CI = 0.78, 2.95; squared coefficient: −2.34, 95% CI = −3.59, −1.01; ΔAIC = 3.55 for quadratic compared to linear model). The line in (b) gives the fitted values from a linear regression that incorporates both a linear and a squared term for the effect of fraction infected on female pupal weight (linear coefficient: 0.71, 95% bootstrapped CI = 0.30, 1.13; squared coefficient: −0.95, 95% CI = −1.42, −0.47; ΔAIC = 3.19 for quadratic compared to linear model). There was no significant effect of viral infection on male pupal weight values (c; neither a linear model nor a model including a squared term fit the data better than a null model; ΔAIC = −2.00 for linear compared to null model; bootstrapped 95 % CI's of both linear and squared coefficients overlapped zero). Error bars in (b) and (c) give ±1 SE for pupal weights.

were larger at the time of virus exposure were more likely to survive. If the negative genetic correlation were due to size-selective effects, we would instead have expected larvae that were smaller at the time of virus exposure to be more likely to survive. Our observation that increased survival is negatively correlated with pupal weight thus appears to be due to a resource allocation trade-off.

The proximal basis of this trade-off may be explained by physiological adjustments in juvenile growth rates which allow the release of resources to support immune system activity. In addition to our results, previous studies suggest that large body sizes at maturity are generally correlated with short developmental times and high reproductive success (e.g. Tammaru et al., 2002). Yet, maximizing the size at maturity requires elevated juvenile growth, especially in animals with determinate growth (Roff, 1992; Dmitriew, 2011). Elevated immunological activity during juvenile stages thus negatively affects adult size and fecundity by utilizing resources that would otherwise be devoted to juvenile growth.

Our results also demonstrate that pathogens can modulate sexual size dimorphism in insects. Specifically, we show that virus exposure has complex sex-specific effects on the relationships between (i) pupal weight and time to pupation, and (ii) pupal weight and infection rate.

Because exposed females pupated at slightly smaller weights for a given pupation time whereas males did not, we conclude that virus exposure interacts with individual sex and growth to affect pupal weights. These sex-specific effects of pathogen exposure strongly affected sexual size dimorphism and resulted in similar pupal weights between late-pupating males and females. In addition to documenting sex-specific effects, our results are consistent with previous work showing that baculovirus exposure affects host development (O'Reilly & Miller, 1989), thus affecting the amount of time available for growth and reproductive investment (Roff, 1992; Klingenberg & Spence, 1997; Day & Rowe, 2002). A deeper understanding of this process requires knowledge of the mechanisms by which sex-specific growth is affected by virus exposure and, in particular, how female investment in reproduction is affected by immune responses to pathogens.

Our results further showed that the effects of virus exposure on pupal weight and sexual size dimorphism vary with pathogen isolate and dose. Specifically, across all pathogen isolates and doses, we found that when mortality was high (i.e. > 50%), both female pupal

weight and sexual size dimorphism declined with increasing mortality. In contrast, when mortality was low (< 50%), female pupal weight and sexual size dimorphism both increased with increasing mortality. We suggest that these nonlinear effects could be explained by two mechanisms. First, at low infection rates, larvae may be displaying a compensatory growth response to virus exposure. That is, following virus clearance, exposed larvae may feed more, allocating the resulting surplus energy to growth (Lee et al., 2006). Second, at high infection rates, resource allocation trade-offs (as discussed above) divert resources from growth to survival, producing smaller female pupal weights. Although compensatory growth mechanisms in response to environmental challenges have been observed across many organisms (Monteiro & Falconer, 1966; Agrawal, 2000; Ali et al., 2003; Korves & Bergelson, 2004; Tammaru et al., 2004; Dmitriew, 2011; Janmaat et al., 2014) , further research is required to test whether this is the correct mechanism. In general, however, these results agree with previous studies showing that female size is especially sensitive to environmental variation (Teder & Tammaru, 2005).

Our results also have implications for our understanding of gypsy moth population dynamics, because trade-offs between fecundity and disease resistance may help explain cycles in gypsy moth abundance over time (Elderd et al., 2008). Gypsy moth outbreak cycles are driven partly by virus epizootics (Woods & Elkinton, 1987), leading to strong but fluctuating selection for resistance. However, given our trade-off results, we expect pupal and egg mass weights to decline after pathogen-induced selection for resistance. Larger egg mass weights may then again be favored in subsequent years when pathogen density in the environment is lower. In addition to contributing to an explanation of outbreak dynamics, this process may also help to explain the maintenance of variation in life-history traits.

Observations from nature have indeed confirmed that egg mass weights decline during population collapses in the gypsy moth (Elkinton & Liebhold, 1990; Myers et al., 2000; Elderd et al., 2008). These observational data, however, have often been explained by effects of forest defoliation or by debilitating or 'sublethal' effects of virus exposure. Under the former hypothesis, high densities of outbreaking insects lead to extensive tree defoliation, insect starvation and consequently the production of small egg masses (Mason, 1974; Elkinton & Liebhold, 1990; Liebhold & Kamata, 2000). In our data, in contrast, female pupal weight varied even though the insects were provided with abundant food. Therefore, we argue that the occurrence of small egg masses in nature is partly due to the fecundity costs of baculovirus resistance, particularly when virus density is high.

Some studies further suggest that debilitating or 'sublethal' effects of pathogen exposure reduce the quality of the maternal environment leading to small egg masses after population crashes (Goulson & Cory, 1995; Rothman & Myers, 1996; Myers et al., 2000; Cory & Myers, 2003, 2009). The literature in this area, however,mostly draws conclusions from phenotypic correlations and has largely discounted the possibility that fluctuations in egg mass weight over time occur through trade-offs arising from genetic effects. Our work demonstrates that egg mass weight can be affected by a negative genetic correlation between survival probability and pupal weight, suggesting that the population dynamics of the gypsy moth may be partly driven by natural selection on disease resistance. Moreover, given that baculoviruses with very similar biology drive outbreaks of other forest Lepidoptera (Moreau & Lucarotti, 2007), our results may be of general significance for understanding insect outbreaks.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Deviance information criterion (DIC) for models testing transgenerational effects on survival probability and pupal weight.

Table S2 Variance partitioning of survival probability, pupal weight and the time to pupation.

Table S3 Model selection for the estimation of the heritabilities of pupal weight and the time to pupation.

Table S4 DIC for models testing among cup correlations for the measured traits.

Figure S1 Experiments conducted on the wild-collected insects to measure survival probability, pupal weight and the timing of pupation in individuals of known relationships.

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