Delayed Virulence and Limited Costs Promote Fecundity Compensation upon Infection

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abstract: Individuals invest limited resources across vital tasks such as reproduction and survival. Individuals can spread reproductive investment over their lifetime, but cues of death or reduced fitness can influence this investment. In some systems, cues of infection induce early but costly reproduction through fecundity compensation as future reproduction becomes uncertain. A key aspect of parasite biology is the delay between exposure to parasites and the onset of virulence. This creates an important window of opportunity for hosts to respond to infection. Existing models have not accounted for this delay or the costs borne by offspring. We combine a theoretical and experimental approach to assess the role of costs and the importance of delay in virulence on fecundity compensation. We find that a delay in virulence selects for plastic fecundity responses even with moderate offspring costs. We tested our model experimentally by exposing pea aphids, *Acyrthosiphon pisum*, to various ecologically relevant cues of infection and monitored lifetime reproduction and survival of these aphids and their offspring. Our challenges induced fecundity compensation, but we did not detect any costs in mothers or offspring. We predict that the relationship between the costs and the delay in onset of virulence, as found here, determines the success of fecundity compensation as an adaptation against parasitism.

Keywords: fecundity compensation, resource allocation, terminal investment.

Introduction

Resources are usually limiting, and an individual has to allocate these limited resources wisely to optimize fitness. Determining the optimal allocation is complicated by the fact that conditions can change and can change quickly. A strategy that spreads out reproductive investment over an individual's lifetime will optimize fitness by trading off reproduction and survival and distributing fecundity across the individual's life span but will suffer if longevity is reduced and that reproductive strategy stays unchanged (Stearns 1992). Many species alter their reproductive strategy, either by accelerating the onset of reproduction or increasing their reproductive effort after cues of increased mortality, for example, from parasite attack. This process is known as fecundity compensation or terminal investment. Fecundity compensation has been described across many invertebrates species (Minchella and Loverde 1981; Thornhill et al. 1986; Polak and Starmer 1998; Adamo 1999; Moret and Schmid-Hempel 2004; Chadwick and Little 2005; Barribeau et al. 2010) but more recently has also been described in vertebrates (Heins 2012) and even bacteria (Poisot et al. 2012).

Immunity, like reproduction, is expensive. These costs can be the result of evolutionary trade-offs (e.g., through negative pleiotropy between increased immunity and other traits; Kraaijeveld and Godfray 1997; McKean et al. 2008), the energy needed to use and maintain immune systems (McKean and Nunney 2001; Ahmed et al. 2002; Jacot et al. 2004), or the self-harm caused by autoimmunity (i.e., cross-reactivity; Sadd and Siva-Jothy 2006). At least in part because of these costs, immunity is imperfect. Even if hosts mount an immune response to a parasite attack, success is never assured. A variety of nonimmunological adaptations have evolved to either reduce the risk of contracting an infection, fight an infection, or ameliorate the damage caused by infection (Parker et al. 2011). The last of these can be achieved by shifting life-history traits to ensure maximal reproductive success through fecundity compensation. These adaptations may be especially important when the likelihood of immune success is low or virulence is high (either through parasite or host mechanisms such as autoimmune damage).

This shift in reproductive investment presumably comes with some costs (Minchella and Loverde 1981; Agnew et al. 2000; Heins 2012). If this adaptation were cost free, then the offspring of an early reproducing individual would dominate the next generation, resulting in evolution toward faster and faster reproduction until reproduction reached its physiological limit. Instead, we see that indi-

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viduals are able to dynamically alter their reproductive investment according to changing conditions that reduce the likelihood of survival. For instance, infected female sticklebacks lay more eggs than uninfected females, but these eggs are smaller (Heins 2012). Similarly, *Biomphalaria glabrata* snails that were exposed to cues of infection laid more eggs than controls after initial exposure but paid for this increase in early egg production with reduced later reproduction (Minchella and Loverde 1981). Pea aphids also accelerate reproduction in response to cues of mortality, such as parasite cues or cutaneous damage (Altincicek et al. 2008; Barribeau et al. 2010). The generality of this type of life-history response and the costs of shifting reproduction are unknown in aphids, since previous studies used few challenges and did not measure costs (Altincicek et al. 2008; Barribeau et al. 2010).

Previous modeling studies have explored under what circumstances differential reproductive investment before and after a challenge can be beneficial (van Baalen 1998; Gandon et al. 2002; O'Keefe and Antonovics 2002; Day and Burns 2003; Bonds 2006; Hall et al. 2007). Mostly, these models assume a negative relationship between host reproduction and survival, such that an increased reproductive effort comes at the cost of reduced longevity. Hosts evolve by altering the fraction of total resources they invest in reproduction compared to survival. Furthermore, hosts are also allowed to plastically change how they invest resources once they are infected. Depending on the effect the parasite has on the host, these models predict different host strategies to evolve. Gandon et al. (2002) showed that when the parasite directly increases host mortality, the optimal strategy of the host is to increase its investment in reproduction upon infection by the parasite. Alternatively, when parasites steal resources from the host, the best strategy of the host is to increase investment in reproduction before the host is infected (Bonds 2006), giving the appearance that the host decreases investment in reproduction postinfection. Thus, the optimal host response qualitatively depends on the effect of the parasite on host resources.

Parasite attacks, however, do not always result in successful infection. For instance, parasitoids may "choose" not to deposit eggs after assessing a host. Furthermore, infectious spores or cells may simply fail to adhere to the host or may arrive in the wrong location within the host and thus fail to establish and invade. Even if the infectious stage of the parasite manages to arrive in the right location, the host immune responses may hinder the successful establishment of the infection. Such a failed infection may alert hosts to the presence of a parasite in addition to further cues of an impending attack, for example, through long-term or induced cues. This exposure of the host to the parasite can inform the host to alter reproductive investment in anticipation of possible attack (Minchella and Loverde 1981; Blair and Webster 2007; Barribeau et al. 2010).

Here we build on resource allocation models used in van Baalen (1998), Gandon et al. (2002), and Bonds (2006) and separate the events of parasite attack and establishment of infection. To account for cues of infection, we allow for hosts to alter their reproductive investment before successful infection. We assume that the effect of infection on the host is a reduction in total available resources. This accounts for both direct effects of the parasite (e.g., theft of resources by the parasite) and general loss of resources (e.g., immune response of the host). We show that our model contains both the Gandon et al. (2002) and Bonds (2006) models as limiting cases when assumptions about the force of infection are valid and can therefore account for both an increase and a decrease of investment in reproduction upon infection. We also consider the scenario where offspring produced under fecundity-compensated resource allocation suffer an intrinsic cost. We then conducted an experiment using pea aphids, *Acyrthosiphon pisum*, to assess whether the costs associated with fecundity compensation in this species are compatible with our model of fecundity compensation and whether different parasite stimuli induce different patterns of fecundity compensation and costs.

Model

We extend a previously published model based on resource-allocation theory (Williams 1966; Bonds 2006) to account for failed parasite attacks and preinfection cues of attacks. We explicitly model only the host population and assume that parasite abundance is sufficiently large such that the attack rate can be assumed constant. A schematic of the model is shown in figure 1*a*. All hosts in the population are born in a susceptible state, *S*. They receive cues of parasite presence through any means (e.g., failed attacks, signaling cues) with a constant rate λ, after which they are considered to be in an exposed state, *E*. These exposed hosts are then successfully infected, *I*, with rate μ. Upon infection, the parasite causes a reduction in available host resources by a factor v. Hosts can reproduce in all three states (susceptible, exposed, and infected), and the offspring are susceptible. The respective reproductive rates of the different states depend on the amount of resources available in each state as well as the allocation of these resources toward reproduction. We assume that hosts can adjust their reproductive rate only once in their lifetime and that they do this once they are exposed. The following system of ordinary differential equations governs the time evolution of the model,

$$\frac{dS}{dt} = (b_S S + b_E E + b_I I)[1 - \kappa(S + E + I)] \quad (1)$$
$$-(d_S + \lambda)S,$$

$$\frac{dE}{dt} = \lambda S - (d_E + \mu)E, \quad (2)$$

$$\frac{dI}{dt} = \mu E - d_I I. \quad (3)$$

Here $1/\kappa$ defines a carrying capacity of the system and can be set to $\kappa = 1$ by rescaling S, E, and I without loss of generality. The birth and death rate functions, b_X and d_X are analogous to those used in Bonds (2006), where $X = S$, E, or I describes the state of the host as either susceptible, exposed, or infected. As in previous models, hosts can trade off investment in reproduction for investment in maintenance and survival (Williams 1966; Gandon et al. 2002; Bonds 2006). We define ρ as the relative increase in investment in reproduction, such that $\rho = e_I/e_S = e_E/e_S$, where e_S, e_E, and e_I are the fraction of resources invested in reproduction in the susceptible, exposed, and infected states, respectively. Setting $e_S = e$ and $e_I = e_E = \rho e$, we get

$$b_S = b_0 e^{\alpha}, \; d_S = d_0(1 - e)^{-\gamma}, \quad (4)$$

$$b_E = b_0(\rho e)^{\alpha}, \; d_E = d_0(1 - \rho e)^{-\gamma}, \quad (5)$$

$$b_I = b_0[(1 - \nu)\rho e]^{\alpha}, \; d_I = d_0[(1 - \nu)(1 - \rho e)]^{-\gamma}, \quad (6)$$

where ν is the fraction of resources stolen by the parasite, b_0 and d_0 are baseline birth and death rates, respectively, and α and γ are coefficients that determine the efficiency of resource conversion to reproduction and survival, respectively. Equivalently to van Baalen (1998), Gandon et al. (2002), and Bonds (2006), we can write the expected lifetime reproductive effort of an individual as

$$w = \frac{b_S}{\lambda + d_S} + \frac{\lambda}{\lambda + d_S} \times \frac{b_E}{\mu + d_E} + \frac{\lambda}{\lambda + d_S} \times \frac{\mu}{\mu + d_E} \times \frac{b_I}{d_I}. \quad (7)$$

The evolutionarily stable strategy (e^*, ρ^*) is such that no other mutant strategy (e', ρ') has higher fitness than (e^*, ρ^*) in a population composed entirely of (e^*, ρ^*) (Smith and Price 1973). For constant attack rates, this is equivalent to finding (e^*, ρ^*) that maximizes the fitness function

$$\left.\frac{\partial w}{\partial e}\right|_{e=e^*,\rho=\rho^*} = 0 \text{ and } \left.\frac{\partial w}{\partial \rho}\right|_{e=e^*,\rho=\rho^*} = 0.$$

We were not able to derive closed-form solutions for the evolutionarily stable strategy (ESS). We, therefore, numerically determine the (e^*, ρ^*) that maximizes the fitness function for a constant attack rate λ. Figure 2 shows ρ^* and e^* as a function of infectivity μ and virulence ν.

As the overall effect of the parasite on the host becomes more severe, that is, with increasing virulence and increasing infection rate, the host increases its pre-exposure investment in reproduction, since the benefits of surviving long enough to risk being infected diminish (fig. 2*a*). The implications of a change in reproductive strategy post-

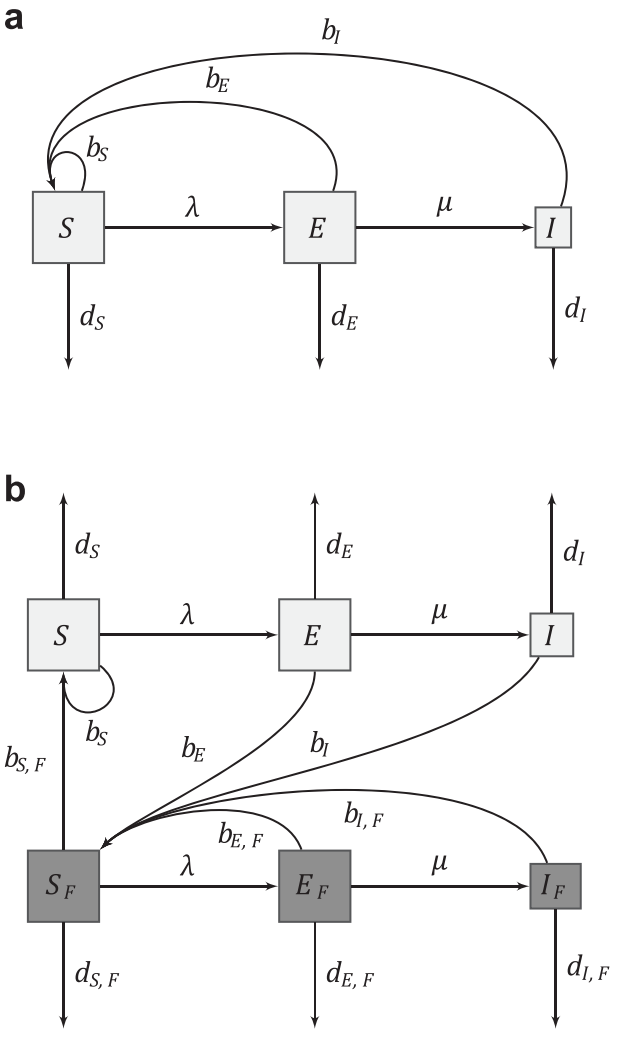

Figure 1: Susceptible-exposed-infected model for resource allocation. *a*, Extension of the Bonds (2006) model to include an exposed class. Individuals reproduce at rates b_x and die at rates d_x, depending on their state and the amount of resources allocated to reproduction ($X \in \{S, E, I\}$). Susceptible hosts are exposed at a rate λ, and infection happens at a rate μ from exposed individuals. The size of the boxes represents the total amount of resources available to the host. *b*, Offspring produced under an adjusted resource allocation scheme pay an intrinsic cost in terms of total available resources. This cost is only carried on for a single generation.

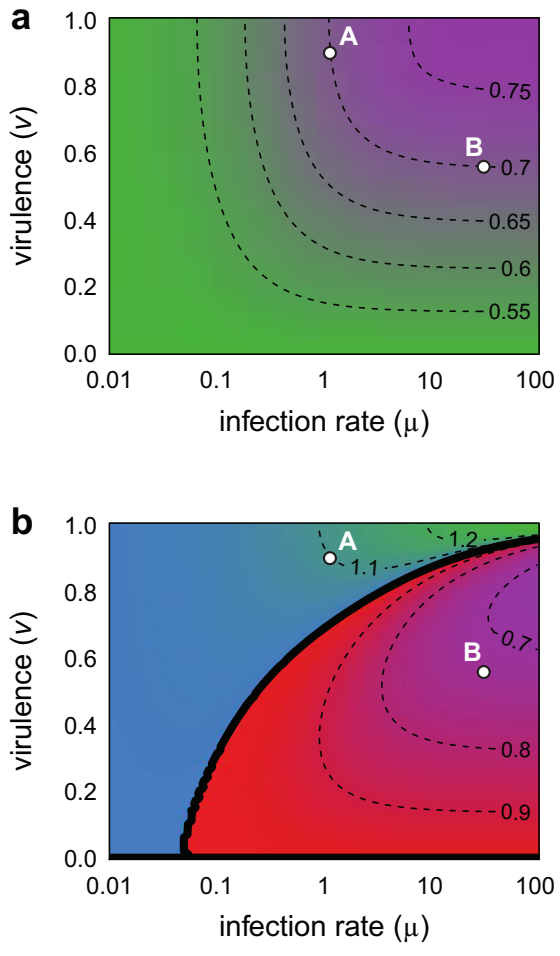

Figure 2: Optimal level of fecundity compensation and pre-exposure investment in reproduction for different values of parasite infectivity μ and virulence v. *a*, The pre-exposure investment in reproduction e. *b*, The shift in reproductive strategy ρ. Red/purple colors signify values of $\rho < 1$, and blue/green colors signify values of $\rho > 1$. For both panels, $b_0 = 10$, $d_0 = 0.1$, $\alpha = 1$, $\gamma = 1$, and $\lambda = 1$.

exposure, however, are qualitatively different depending on virulence v and infection rate μ (fig. 2*b*). Individuals alter their reproductive strategy upon exposure in our model. Once exposed, the optimal strategy for the remainder of an individual's life is independent of the reproductive strategy pre-exposure. The benefit of investing in reproduction over survival then depends on the balance between time spent in the exposed phase versus time spent in the infected phase. The implications of balancing the time spent in each phase can be understood by considering two limiting cases: (i) When v is large and μ is moderate, then the optimal strategy is to increase investment in reproduction upon exposure (fig. 2, point A). Since v is large, the parasite steals most of the resources from the host, and therefore the host can survive in the infected phase for only a short time. In the limit $v \to 1$, the parasite steals all resources from the host and the host immediately dies upon the establishment of infection. This can be viewed as an additional mortality rate for exposed individuals, and the model becomes formally equivalent to the model in Gandon et al. (2002). In this case, the postexposure investment in reproduction is always larger than the pre-exposure investment (see app. A [apps. A–C available online] and Gandon et al. 2002). (ii) When v is moderate and μ is large, then the optimal strategy is to decrease investment in reproduction (fig. 2, point B). A large μ means that successful infection follows quickly after an attack or other cue of impending attack. In the limit $\mu \to \infty$, the model become formally equivalent to the model in Bonds (2006). In this case, the postexposure investment in reproduction is always smaller than the pre-exposure investment (see app. A; Bonds 2006).

In many host-parasite systems, the dynamics of the parasite population depend on the host population and vice versa. This is often taken into account through an attack rate λ that is proportional to the number of infected individuals (i.e., density- and frequency-dependent transmissions) or proportional to the number of dying individuals (i.e., parasites are released in a burst when an individual dies). While the inclusion of such feedback loops may give quantitatively different results, the two limiting cases still yield two qualitatively different strategies (increase/decrease in investment in reproduction) after exposure for high virulence/low infection rate and low virulence/high infection rate (see app. B).

Offspring Cost Model

So far, we have considered the case where there is a perfect trade-off between reproduction and investment (with the conversion and efficiency coefficients α and γ). Sudden changes in resource allocation may be more energetically expensive than continuing with resource use as before exposure or infection. This, in turn, may come with overhead costs that manifest either directly to the host or through the quality of produced offspring. We thus consider the case where offspring produced under a plastically altered resource allocation scheme have fewer resources than the offspring produced normally (fig. 1*b*). We also assume that the reduction in resources, φ, is proportional to the magnitude of the change in resource allocation,

$$\varphi(\rho) = \exp\{-\eta|e(\rho - 1)|\}, \tag{8}$$

where η is a scaling factor for the strength of the reduction in offspring resources. The birth and death rates for the offspring cost (oc) model are then

$$b_S^{oc} = b_0(\varphi e)^{\alpha},\ d_S^{oc} = d_0[\varphi(1-e)]^{-\gamma}, \quad (9)$$

$$b_E^{oc} = b_0(\rho\varphi e)^{\alpha},\ d_E^{oc} = d_0[\varphi(1-\rho e)]^{-\gamma}, \quad (10)$$

$$b_I^{oc} = b_0[(1-v)\rho\varphi e]^{\alpha},\ d_I^{oc} = d_0[(1-v)\varphi(1-\rho e)]^{-\gamma}. \quad (11)$$

When $\eta = 0$, then $\varphi = 1$, and therefore we recover the model without offspring costs. Equivalently, when $\eta \to \infty$, then $\varphi \to 0$, and the produced offspring have zero resources.

As η increases from zero, so does the fitness cost of the produced offspring. Any benefit gained from altering the reproductive strategy thus becomes less important (fig. S1; figs. S1–S8 are available online in the supplementary PDF). A shift in reproductive strategy remains beneficial as long as costs to the offspring are modest.

Experimental Test Using *Acyrthosiphon pisum*

Our model predicts that flexible responses in investment in reproduction can evolve if costs are limited. Furthermore, the direction of this shift depends on the virulence of the parasite and whether reliable cues of impending infection are available. We expect to find an increase in reproduction after exposure in systems where, on the one hand, virulence is high and, on the other hand, not all attacks lead to successful infections or where other cues of impending attack are present and reliable.

To assess this, we experimentally exposed pea aphids, *Acyrthosiphon pisum*, to various cues of parasites and measured lifetime reproduction and longevity of both the test individuals and their daughters to also capture additional costs borne only by the induced offspring. *Acyrthosiphon pisum* shift in reproduction in response to some cues (Altincicek et al. 2008; Barribeau et al. 2010), but the costs associated with this shift remain unknown. Given their explosive reproductive potential, we were interested in why these insects retain a plastic response and reproduce more slowly than their maximum.

Experimental Methods

We exposed pea aphids, *A. pisum*, from three clonal lines (A06, 5A, LSR1) to one of five treatments. Two of these lines are commonly used lab lines that originated from the United States; 5A was collected from Wisconsin in 1999 and LSR1 from New York in 1998. The aphid line A06-01 (hereafter referred to as A06 for brevity) was collected from Steinmaur, Zurich, Switzerland, in 2006. The LSR1 line originally harbored the secondary symbiont *Regiella insecticola* but was experimentally cleared of secondary symbiont infection (International Aphid Genomics Consortium 2010). The 5A aphids were collected without secondary symbionts (Oliver et al. 2003). We confirmed that the local aphid line was not infected with *R. insecticola*, *Hamiltonella defensa*, *Serratia symbiotica*, *Rickettsia*, *Spiroplasma*, or the X-type symbiont by polymerase chain reaction following the methods described in Ferrari et al. (2012).

We kept the aphids asexually on fava bean seedlings (*Vicia faba*) under 16-h light cycles in a climate chamber set at 20°C. We challenged 10-day-old aphids by stabbing their dorsal abdomens with a size 00 insect needle contaminated with the heat-killed culture of a gram-negative (G−) bacterium (*Enterobacter cloacae*), an aphid pathogenic unnamed gram-positive (G+) bacterium, or the fungus (F) *Erynia neoaphidis*; others we stabbed with a sterile (S) needle as a wounding control or left untreated (baseline, BL) as naive controls. We chose 10-day-old aphids as, by this time, all of these aphids should be capable of reproducing. Pea aphids are usually reproducing by 7–9 days old (S. M. Barribeau, personal observation). We standardized the challenge innocula to an optical density of OD600 = 0.5 suspended in Ringer's solution. The two bacterial species were cultured from sick aphids and kill healthy aphids when experimentally infected (Gerardo et al. 2010; Laughton et al. 2011). The fungus is an aphid parasite and was obtained from the USDA entomopathogen culture collection. We assigned 10 aphids from each line to each treatment (150 aphids total). After treatment, we kept aphids singly in a 35-mm-diameter petri dish on a leaf with wet filter paper beneath. We changed the leaves every 2 days and kept the aphids in the same light and temperature regime as above. We monitored survival every day for days 1 to 11 after challenge and every 2 days after that, and we recorded fecundity every 2 days for the duration of the experiment. We monitored one daughter that was born 1 day after challenge or sham treatment for each aphid to explore possible fitness differences in the offspring produced under fecundity compensation. We recorded mass at day 1, time until first reproduction, the number of offspring every 2 days, and longevity of these F_1 daughters. All collected data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4md5s (Leventhal et al. 2014).

Statistical Methods

Reproductive Effort. To test whether the challenges have an effect on the reproductive output of the aphids, we fit a parametric model of reproduction using a likelihood-based approach. This allows us to use the complete reproduction data in a single step. The different treatment groups can then be compared on the basis of the parameterized lifetime reproductive function.

Such an approach has advantages over other methods. Comparing early reproduction and late reproduction re-

quires the arbitrary definition of "early" (previous to a certain time point) and "late" (subsequent to a certain time point), and the choice of when to split the time has a strong influence on the outcome of a statistical test. A day-by-day comparison of reproductive output would require multiple tests using only part of the data, thereby decreasing the power of each individual test.

We assume that after maturation, aphids continuously produce offspring at a rate that decreases over the life span of the aphid. We model this decrease using three different functional families (linear, exponential, and logistic decreases; fig. 3) and assess the statistical fit of these models using both the deviance information criterion (DIC; Gelman et al. 2004) and the Bayesian predictive information criterion (BPIC; Ando 2007). We control for overdispersed data by comparing model fits of Poisson and negative binomial distributed daily offspring counts. Goodness of fit of the model is determined using the posterior predictive distribution in the Bayesian framework.

Using the fitted parametric model, we calculate the expected difference in offspring at various times postchallenge between the treatment groups and the naive control group to test for significant differences in daily reproductive output due to treatment. A detailed description of these statistical methods is given in appendix C.

Survival. We performed a survival analysis on the aphids using semiparametric Cox proportional hazard regression and a parametric survival regression model with a Weibull hazard implemented in the R survival package (Therneau 2012). Cox regression assumes a baseline hazard function, that is, death rate, and infers the relative increase or decrease in death rate proportional to the baseline. We controlled for nonproportionality using a proportional hazards test. We used aphid line and treatment as covariates to see if the different challenges had different effects on longevity of the aphids.

Offspring Weight. We analyzed daughter mass with an ANOVA using line and treatment as fully crossed fixed effects. All of the above analyses were performed in R (R Development Core Team 2012).

Experimental Results

Mothers. We first assessed how aphids alter their reproductive output after challenge and how this shift in reproductive strategy influences lifetime reproduction, survival, and hence fitness.

Reproduction. The logistic reproduction function was the best fit to the data. The DIC and BPIC values for the different model fits (no effect, line effect, treatment effect, line/treatment interaction) are shown in table 1 for the

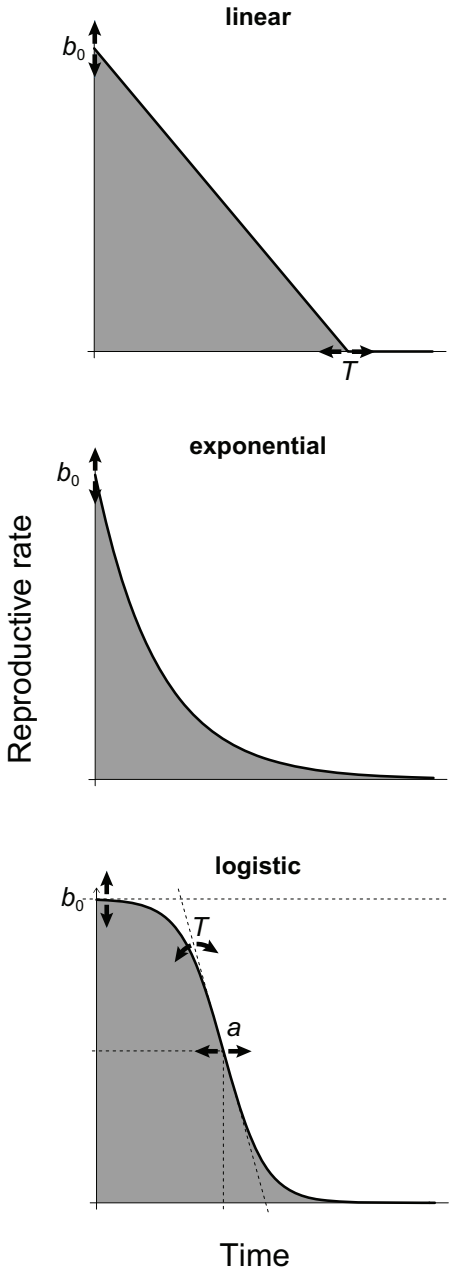

Figure 3: Reproductive rate functions: linear (*top*), exponential (*middle*), and logistic (*bottom*). The shapes of the linear and exponential functions are changed by two parameters, b_0 and T. The shape of the logistic function is changed by three parameters: b_0, T, and a. Parameter b_0 increases the baseline reproductive rate for all three functions, and parameter T determines the rate of decay. In the logistic function, a is time of the inflection point, where the actual reproductive rate is half of the baseline rate. The shaded area below the curve represents the total lifetime reproductive output, given that the aphid does not die, R_y.

Table 1: Deviance information criterion (DIC) and Bayesian predictive information criterion (BPIC) for determining model fit for the logistic birth function

	Model	DIC	BPIC	P	p_D
(1) No stratification	Poisson	4,418	4,421	3	2.96
(2) Treatment only	Poisson	4,344	4,359	15	14.81
(3) Line only	Poisson	4,243	4,251	9	7.68
	Negative binomial	4,161	4,173	12	11.7
(4) Treatment and line	Poisson	4,130	4,170	45	40.21
	Negative binomial	4,104	4,152	60	47.8
	Mixed	4,097*	4,140*	48	42.7

Note: The DIC takes into account both goodness of fit as well as model complexity. The BPIC is a more conservative measure with a higher penalty for more complex models. The full model with separate coefficients for all line-treatment combinations and overdispersed data (negative binomial) yields the lowest DIC, whereas the line-only model has the lowest BPIC. Overall, a model that allows for overdispersion in only three of the line-treatment combinations has both the lowest DIC and the lowest BPIC. The real number of parameters, p, as well as the effective number of parameters, p_D, are indicators of model complexity. The mixed model allows for overdispersion in three of the line-treatment combinations: 5A/gram-negative, 5A/sterile, and A06/gram-positive. The asterisk indicates the model that is selected for on the basis of the DIC and BPIC, respectively.

logistic birth function and tables S1, S2 (in the supplementary PDF) for the exponential and linear birth functions, respectively. In all cases, the logistic rate function better described the data than either the exponential or the linear classes (fig. 3). This is consistent with the good visual fit provided by the logistic rate function (see figs. 4, S5, S6). Within all three rate function classes, the full model with different rate parameters for both line and treatment gave the best fit. This indicates that the effects of treatment and aphid line are independent of the shape of the rate function. The posterior distribution of the parameters is shown in figure S2.

For all treatments and lines, the responses to sterile stabbing yielded large highest-probability density intervals for all of the model parameters. This suggests that there was no homogeneous response to the sterile stabbing across animals. Naive untreated aphids (BL) from lines 5A and LSR had similar reproductive functions. The corresponding aphids from line A06 displayed a markedly lower baseline reproductive rate, b_0, while sustaining this reproduction for a longer period (larger a, later inflection point). Overall, aphids from line A06 showed the strongest reproductive increase in response to the challenges. These aphids responded to the treatments by increasing b_0 and decreasing a and thus increased their baseline reproductive rate but remained at this higher rate for less time before reproduction stopped. We did not observe a strong difference in the rate of reproductive decay (T) between naive and challenged aphids. Aphids from 5A and LSR did not show a strong response to any of the challenges.

All treatment groups of A06 aphids respond by increasing their early reproductive output relative to the naive control aphids, with the maximum difference occurring at around day 15 (fig. 5). After this shift, the reproductive output of these aphids slows, and by day 20 we no longer see any significant differences between the treatment and baseline aphids. There is also no significant difference in the total lifetime reproductive output of all treatment groups of the A06 line (day 35), suggesting that any increase in reproduction upon challenge is paid for with reduced later reproduction.

For aphids from lines 5A and LSR, all challenges (except LSR/G−) result in a lower lifetime reproductive output. In 5A, this reduction manifests itself already from day 4 for the sterile challenge group. Daily reproductive output in the other treatment groups does not significantly differ from the baseline until around day 15. Relative to the sterile challenge rather than the baseline, aphids from all other treatment groups have a higher reproductive output early on, but differences in lifetime reproductive output are significant only for the gram-positive (5A/G+) treatment (fig. 5*c*).

The gram-positive challenge has the largest effect on LSR aphids. The response to the fungal (F) challenge in these lines resembles the sterile challenge, and we do not see any significant change in reproductive output for gram-negative-challenged aphids.

Survival. All challenges reduced the survival of the aphids. The Cox proportional hazards likelihood ratio test showed significant effects of both treatment ($\chi^2 = 16.0$, df $= 4$, $P = .003$) and line ($\chi^2 = 8.10$, df $= 2$, $P = .017$). Aphids from line 5A survived longest, followed by aphids from line A06 (hazard ratio $h_{A06} : h_{5A} = 1.69$, 95% confidence interval [CI]: 1.11–2.55; $z = 2.475$, $P = .013$) and aphids from the LSR line (hazard ratio $h_{LSR} : h_{5A} = 1.65$, 95% CI: 1.10–2.48; $z = 2.396$, $P = .017$). The sterile

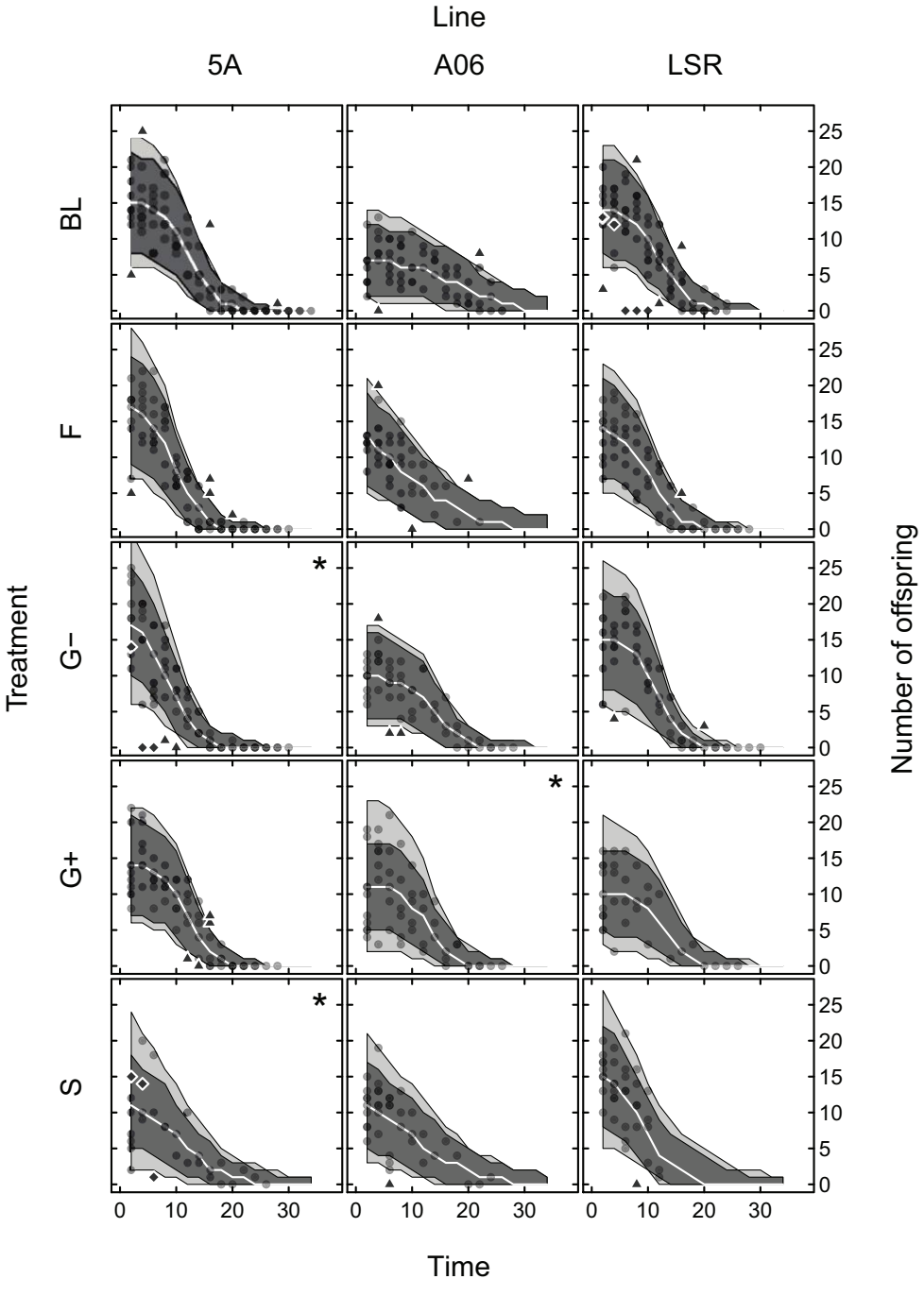

Figure 4: Posterior predictive check of the Poisson and negative binomial models. Rows are aphids from the different treatments: baseline (BL), fungal (F), gram-negative (G−), gram-positive (G+), and sterile (S) treatments. The dark and light shaded areas show the number of offspring within the 95% highest probability density (HPD) interval for 10,000 samples from the posterior predictive distribution for the Poisson model and negative binomial model, respectively. The triangles are measurements that lie outside of both HPD intervals and can be considered outliers. Three aphids were excluded from the fitting due to abnormal values identified post hoc (diamonds). Only three line-treatment groups displayed evidence for overdispersed data: 5A/G−, 5A/S, and A06/G+ (indicated by an asterisk; lower deviance information criterion and Bayesian predictive information criterion for the negative binomial model; see supplementary materials, available online).

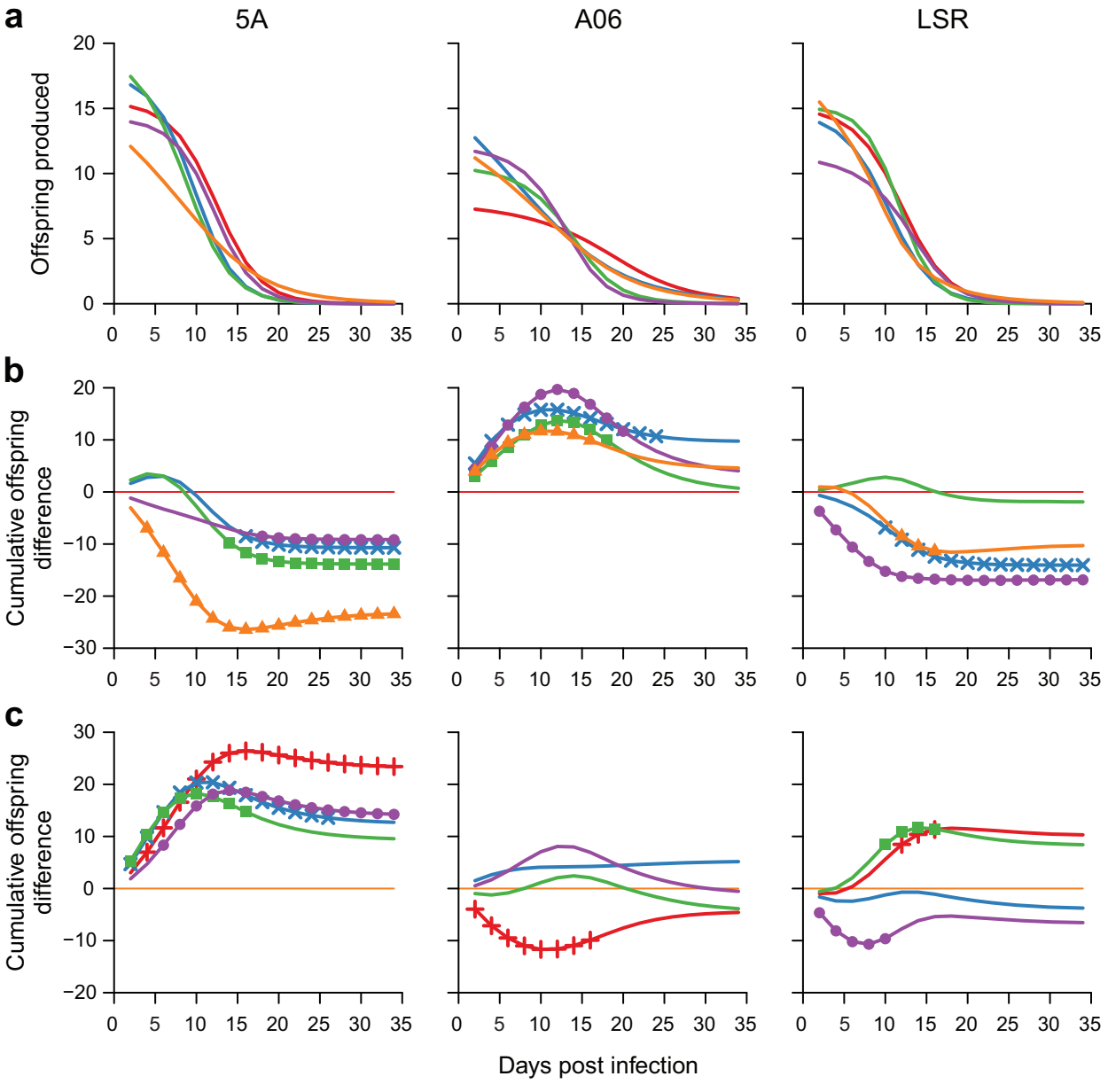

Figure 5: Expected reproductive output of mothers. *a*, Mean reproductive output every 2 days over 10,000 samples from the posterior. *b*, Difference in expected cumulative number of offspring between the treatment groups and the control groups of the three different lines. Positive (negative) values at a given day indicate that aphids from a treatment group have produced more (less) offspring up to that day than aphids from the baseline group of the same line. A symbol on the curve at a given day *t* indicates that the difference in cumulative number of offspring between a treatment group and the baseline group at day *t* are significant (posterior probability >0.95). *c*, Difference in expected cumulative number of offspring between the treatment groups and the sterile controls of the three different lines. Baseline: red (plus signs); fungal: blue (multiplication crosses); gram-negative: green (squares); gram-positive: purple (circles); sterile: orange (triangles).

stab had the strongest effect of all treatments, increasing the hazard by 2.75 (95% CI: 1.61–4.70; $z = 3.71$, $P = .00020$) compared to untreated aphids. Aphids challenged with gram-positive bacteria displayed an increase in hazard of 1.93 (95% CI: 1.15–3.27; $z = 2.467$, $P = .014$). We did not find an effect in the fungal (hazard ratio = 1.45 [95% CI: 0.865–2.44]; $z = 1.41$, $P = .158$) and gram-negative challenges (hazard ratio = 1.34, 95% CI: 0.7970–2.243; $z = 1.100$, $P = .271$). Since there was no evidence for nonproportionality both globally and for each of the

covariates, the Cox proportional hazards analysis is appropriate. The survival of aphids from different lines did not respond differently to the different treatments ($\chi^2 = 5.33$, df = 8, $P = .722$).

The parametric regression model with Weibull-distributed hazard function, including both treatment and line effects, best described survivorship based on the corrected Akaike Information Criterion (AICc) for small sample sizes. The AICc values were: no effect = 1,035, treatment only = 1,030, line only = 1,034, treatment + line = 1,030, and treatment/line interaction = 1,045. The AICc already corrects for model complexity, such that these results are consistent with the Cox proportional hazards model with a strong treatment effect and a weaker line effect. The survival curves within aphid lines are shown in figure S3.

Daughters. We next assessed to what extent offspring from mothers with altered reproductive investment pay a cost in terms of lifetime reproductive output.

Weight at birth. Aphid lines differed significantly in offspring weight ($F_{2, 122} = 39.4$, mean square error [MSE] = 0.419, $P = 6.07 \times 10^{-14}$); treatment did not alter offspring weight ($F_{4, 122} = 1.54$, MSE = 0.00163, $P = .195$). Tukey's honestly significant differences tests reveal that there are significant differences between all lines: Δ(A06, 5A) = 0.0602 ($P = 1.40 \times 10^{-13}$), Δ(LSR, 5A) = 0.0188 ($P = .016$), and Δ(LSR, A06) = 0.0414 ($P = 6.63 \times 10^{-8}$). The lines that reproduce fastest (5A and LSR) also have the largest offspring (see also fig. S4).

Reproduction. There was discrepancy between the two model selection criteria for the reproductive output of the daughters. The model with separate birthrate function for all of the line-treatment combinations and overdispersion in five line/treatment groups (A06/F, LSR/BL, LSR/F, LSR/G−, and LSR/S) yielded the lowest DIC value ($DIC_{4, mixed}$ = 4,763; table 2). The BPIC selects for the model with a line effect and overdispersion ($BPIC_{2, NB}$ = 4,794). When examining the within-line effects, we see that the BPIC selects for the model with treatment effect and overdispersion in some of the groups for lines 5A and A06 but for no effect of treatment in line LSR ($BPIC_{4, mixed}$ [5A] = 1,658, $BPIC_{4, mixed}$ [A06] = 1,243, $BPIC_{4, mixed}$ [LSR] = 1,905, $BPIC_{4, NB}$ [5A] = 1,666, $BPIC_{2, NB}$ [A06] = 1,234, $BPIC_{2, NB}$ [LSR] = 1,895). This is reflected in the cumulative offspring difference compared to the baseline aphids (fig. 6), where we see significant effects in daughters of 5A/F mothers and daughters of A06/G− mothers. There is no significant effect of the mother's treatment on total lifetime reproductive output of daughters (day 43).

Survival. Survivorship varied only with line ($\chi^2 = 8.56$, df = 2, $P = .014$) not with the mothers' treatment ($\chi^2 = 5.48$, df = 4, $P = .241$) or line-treatment interactions ($\chi^2 = 3.80$, df = 8, $P = .874$). Line A06 had an increased hazard of 2.05 over line 5A ($z = 2.81$, $P = .005$), and line LSR has an increased hazard of 1.46 over line 5A ($z = 1.79$, $P = .073$).

Discussion

Altering reproduction upon infection, cues of infection, or any other reliable cues of death may be highly important to host fitness. When resistance is possible and virulence is low, reducing investment in costly reproduction may provide more resources with which to mount an immune response. When resistance is futile, because either resistance is impossible or virulence is high, hosts may still compensate by reproducing early and forgoing a costly immune battle. We explored the conditions under which fecundity compensation is likely to occur, in particular focusing on when individuals may have reliable cues of impending death and the impact of potential costs of such a strategy.

In many host-parasite systems, there is an inherent delay between parasite exposure and the onset of virulence. We extended a previous model for fecundity compensation to account for preinfection cues and moderate costs of shifting reproduction in response to cues of death. Our model can lead to different evolutionary outcomes, depending on the virulence of the parasite as well as the rate of successful infection. When virulence is high but the rate of successful infection is low, it is evolutionarily favorable to evolve a plastic response to parasite attacks, where an individual redirects resources into reproduction and away from maintenance. When virulence is low and the rate of successful infection is high, then the optimal plastic response of an individual should be to decrease investment in reproduction upon attack but increase preattack reproductive output. These two cases have been specifically modeled in Gandon et al. (2002) and Bonds (2006), respectively, and our model recovers these opposing models as limiting cases.

Apart from suboptimal resource allocation, other costs could also be associated with a shift in reproductive strategy. These costs may affect the individual itself but may also be borne by the offspring. By incorporating such costs into our model of resource allocation, we have shown that plastic responses can remain beneficial, as long as the costs to the offspring are moderate. We used pea aphids to measure any detectable costs in mothers and daughters that are associated with reproductive shifts in response to parasite cues and to assess how these aphids alter their reproductive output in response to these different cues.

We found considerable variation in reproductive output and in the response to challenges across three aphid clones. In the aphid line A06, we found that individuals increase

Table 2: Deviance information criterion (DIC) and Bayesian predictive information criterion (BPIC) for determining model fit of daughters' reproductive output

	Model	DIC	BPIC	P	p_D
(1) No stratification	Poisson	4,839	4,843	3	3.03
(2) Treatment only	Poisson	4,836	4,851	15	15.0
(3) Line only	Poisson	4,811	4,820	9	8.98
	Negative binomial	4,784	4,794*	12	10.2
(4) Treatment and line	Poisson	4,799	4,840	45	41.1
	Negative binomial	4,782	4,828	60	45.5
	Mixed	4,763*	4,807	50	43.8

Note: The real number of parameters, p, as well as the effective number of parameters, p_D, are indicators of model complexity. The mixed model allows for overdispersion in five of the line-treatment combinations: A06/fungal, LSR/baseline, LSR/fungal, LSR/gram-negative, and LSR/sterile. The asterisk indicates the model that is selected for on the basis of the DIC and BPIC, respectively.

their reproductive output immediately after being challenged and that this increase was independent of the class of parasite (fungal or bacterial) or even when injured without the presence of a parasite. The total lifetime reproductive output of these aphids, however, was equal to that of the unchallenged ones. Thus, viewed over the complete life span of the aphids, there is no difference in the total allocation of resources into reproduction but rather an accelerated rate of reproduction due to the anticipation of early death. These early induced offspring were paid for by reduced later reproduction. It is worth noting that if our challenges had been virulent and the aphids had died at a rate that is normal for pea aphids given live parasites (within 2–7 days; Gerardo et al. 2010; Laughton et al. 2011; S. M. Barribeau, personal observation), then these early reproducing aphids would have had substantial fitness advantage over any others that failed to respond with earlier reproduction.

In the 5A line, all challenges resulted in a reduced lifetime reproductive output. These aphids appear to redirect resources from reproduction to maintenance as a response to the injury. Compared to the sterile injury, we did observe a significant increase in early reproductive output for aphids that were challenged with parasites. Thus, even though total lifetime reproductive output is reduced as a result of injury, the presence of a parasite induces an additional reallocation of resources that brings reproductive output back to the level of unchallenged aphids. This enhanced reproductive output is sustained for only the first 15–20 days for fungal and gram-negative challenges, comparable to the timescales observed in the A06 aphids.

Overall, aphids from the 5A and LSR lines, however, had a much higher baseline reproduction than the A06 aphids. The 5A and LSR lines are laboratory lines that have likely been exposed to selection for rapid reproduction over the past decade of laboratory culture. The A06 line was more recently collected from the field. The 5A and LSR lines may have evolved to reproduce at their maximum capacity, such that an increase in reproductive output is no longer possible, but resources can be reallocated to bring reproductive output back to the levels of the baseline. A pronounced reproductive shift was not found in aphids from the LSR line, which responded differently to each of the different challenges.

We also found that survivorship is influenced by the challenge. All challenges reduced the lifetime of the aphids. Surprisingly, this reduction was most severe for aphids that received the sterile challenge. Sterile injury in another study of pea aphids also reduced reproduction more severely than exposure to bacterial cues, although that study did not measure longevity (Barribeau et al. 2010). Cuticular insult alone may be sufficiently damaging to reduce aphid survivorship, but the absence of antigenic cues might not induce the fecundity response and perhaps even the wound-healing or immune responses that other challenges produce. Pea aphids have a dramatically diminished immune repertoire as they lack common recognition molecules (peptidoglycan recognition proteins), the majority of the immune deficiency pathway, or most known antimicrobial peptides (Gerardo et al. 2010). Despite this, pea aphids produce both cellular and humoral responses to experimental challenges like those used in this study (Laughton et al. 2011). The molecular mechanisms that pea aphids use to identify and respond to parasites are still unknown, although some likely candidates may be the beta-glucan receptor proteins (BGRPs, a.k.a. gram-negative binding proteins) or other recognition genes such as Dscam or hemomucin.

Our experimental results confirm that pea aphids appear able to respond to cues of impending risks by increasing reproduction and that the costs of shifting reproductive investment are minimal to both the mother and her off-

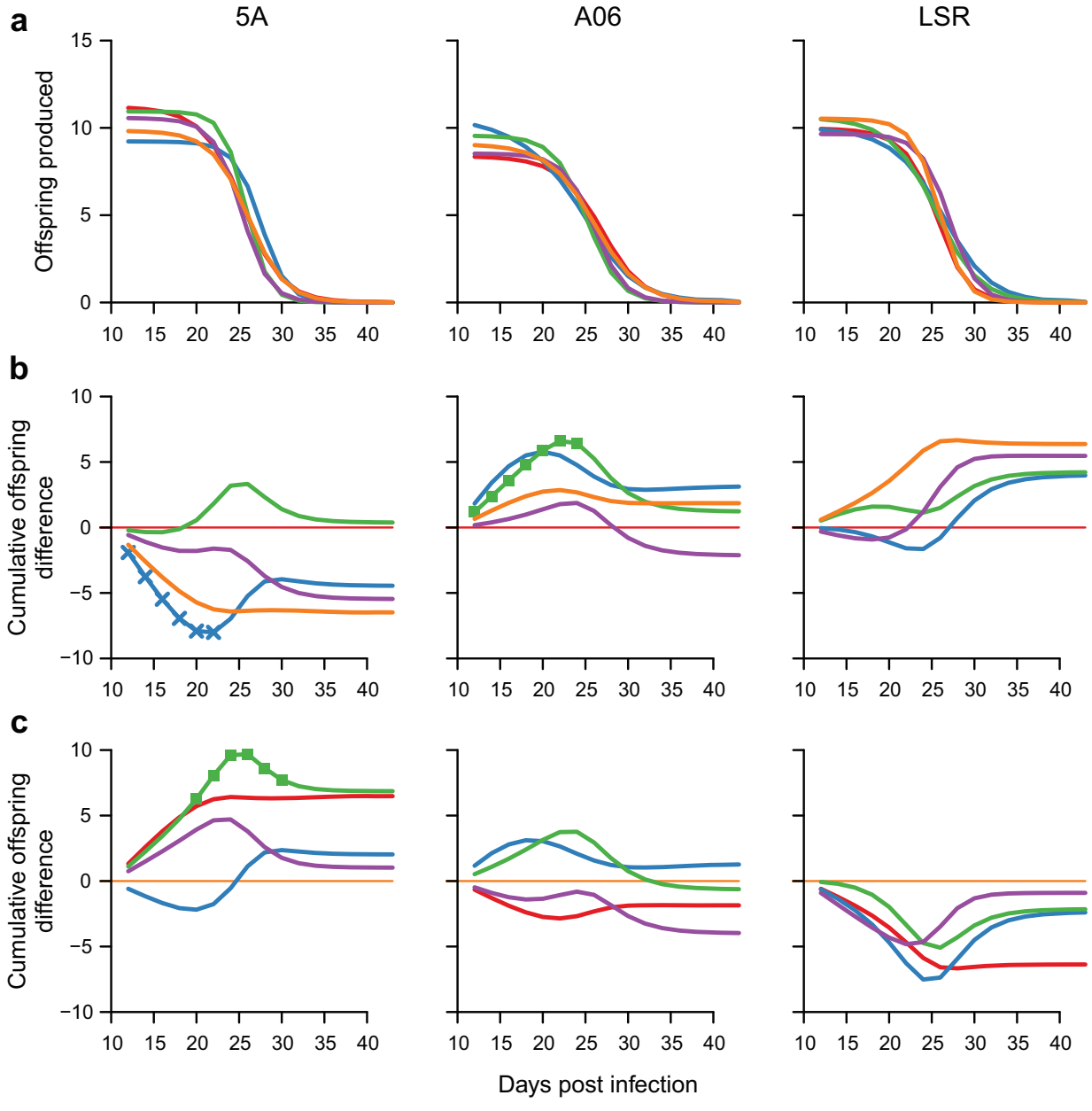

Figure 6: Expected reproductive output of mothers. *a*, Mean reproductive output every 2 days over 10,000 samples from the posterior. *b*, Difference in expected cumulative number of offspring between the treatment groups and the control groups of the three different lines. Positive (negative) values at a given day indicate that aphids from a treatment group have produced more (less) offspring up to that day than aphids from the baseline group of the same line. A symbol on the curve at a given day *t* indicates that the difference in cumulative number of offspring between a treatment group and the baseline group at day *t* are significant (posterior probability >0.95). *c*, Difference in expected cumulative number of offspring between the treatment groups and the sterile controls of the three different lines. Baseline: red (plus signs); fungal: blue (multiplication crosses); gram-negative: green (squares); gram-positive: purple (circles); sterile: orange (triangles).

spring in this system. Our model predicts that such behavior is expected when virulence is high and the rate of successful infection is low compared to cues of attack. Virulence of the tested parasites is generally high in pea aphids (Milner 1982; Gerardo et al. 2010; Laughton et al. 2011). Apart from direct challenges, as in our experiment, other cues that could trigger fecundity compensation are the probing behavior of parasitoids (some parasitoids assess the suitability of potential hosts through attacks but do not necessarily lay their eggs), direct encounters of

parasites (e.g., failed parasitoid attacks, encountering the aphid mummies produced by successful parasitoid infection, or sporulating carcasses), or signaling by other attacked aphids with alarm pheromones such as (*E*)-β-farnesene (Pickett et al. 1992). Thus, cues of infection are common in relation to successful infection. In addition, aphids that have received reliable cues may be able to better respond with fecundity compensation than the aphids measured in our experiment because many of these cues are not costly themselves.

Early reproduction in a rapidly reproducing clonal species such as pea aphids seems to be an unbeatable strategy, as any mutants producing early and many offspring would rapidly dominate a population. Despite this obvious advantage, some lines seem to have flexible reproductive schedules that allow a plastic response to cues of impending death. We explored how aphids respond to cues of a single cause of mortality, that is, parasite attack, but there are a variety of other uncertainties that aphids will face in ecological settings. These include declining plant quality or availability, predation, and unfavorable abiotic changes such as temperature fluctuations. Any of these additional stressors could also produce comparable shifts in the timing of reproduction and may explain the variability of responses to our challenges. Wild aphids likely do not reproduce at their maximal rate to hedge their bets against uncertainty in their total environment, which harbors a myriad of changeable conditions that will reduce their future fitness.

In this article, we have focused on only the host population. The ability to sense a heightened risk of death and change one's resource allocation strategy could have interesting coevolutionary consequences. Changes in life-history traits that increase host abundance can in turn be favorable for the parasite. A full coevolutionary analysis of such a host-parasite system would allow for better understanding of how the optimal host response feeds back into the evolution of parasite virulence and infection rate. This may offer new insight into the evolution of plastic life-history traits in host-parasite systems.

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