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# The target of selection matters: An established resistance—development-time negative genetic trade-off is not found when selecting on development time

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## Abstract

Trade-offs are fundamental to evolutionary outcomes and play a central role in eco-evolutionary theory. They are often examined by experimentally selecting on one life-history trait and looking for negative correlations in other traits. For example, populations of the moth *Plodia interpunctella* selected to resist viral infection show a life-history cost with longer development times. However, we rarely examine whether the detection of such negative genetic correlations depends on the trait on which we select. Here, we examine a well-characterized negative genotypic trade-off between development time and resistance to viral infection in the moth *Plodia interpunctella* and test whether selection on a phenotype known to be a cost of resistance (longer development time) leads to the predicted correlated increase in resistance. If there is tight pleiotropic relationship between genes that determine development time and resistance underpinning this trade-off, we might expect increased resistance when we select on longer development time. However, we show that selecting for longer development time in this system selects for reduced resistance when compared to selection for shorter development time. This shows how phenotypes typically characterized by a trade-off can deviate from that trade-off relationship, and suggests little genetic linkage between the genes governing viral resistance and those that determine response to selection on the key life-history trait. Our results are important for both selection strategies in applied biological systems and for evolutionary modelling of host–parasite interactions.

KEYWORDS

baculovirus, costs, defence, experimental evolution, immunity, infection, insect, life-history, *Plodia interpunctella*, Trade-off

Lewis J Bartlett and Elisa Visher equally contributed to this work.

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## 1 | INTRODUCTION

Trade-offs remain fundamental to modern ecological and evolutionary thinking (Acerenza, 2016; Shoval et al., 2012). More specifically, they are central to our understanding of the evolution and ecology of infectious diseases (Alizon, Hurford, Mideo, & Van Baalen, 2009; Alizon & Michalakis, 2015; May & Anderson, 1983) and to resistance evolution to both chemical and biological pressures (Best & Hoyle, 2013; Best, Webb, White, & Boots, 2011; Boots & Bowers, 1999; Boots & Haraguchi, 1999; Foster, Denholm, Poppy, Thompson, & Powell, 2011; Gandon, van Baalen, & Jansen, 2002; Gandon, Buckling, Decaestecker, & Day, 2008; Gandon & Vale, 2013; Gemmill & Read, 1998; Gillespie, 1975; Gwynn, Callaghan, Gorham, Walters, & Fellowes, 2005; Miller, White, & Boots, 2007; Sheldon & Verhulst, 1996; Shirley & Sibly, 1999). This large body of theoretical and empirical work has made substantial progress in our fundamental understanding of evolutionary trade-offs, but there remains the need to better integrate theory and empirical findings (Cressler, Graham, & Day, 2015; McKean, Yourth, Lazzaro, & Clark, 2008; Schmid-Hempel, 2003). A clear understanding of fundamental evolutionary processes is crucial to the evolutionary management of resistance in pest and pathogen control (e.g. Brosi, Delaplane, Boots, & de Roode, 2017; Brown, 2002; Seifi, Visser, & Bai, 2013; Yan, Severson, & Christensen, 1997), and therefore, investigating the fundamental nature of trade-offs with both empirical and theoretical studies remains of considerable importance.

Theory generally implicitly assumes that trade-offs are symmetrical such that selection on either trait results in a response in the other (Abrams, 2001; Geritz, Kisdi, Meszéna, & Metz, 1998; Kisdi & Geritz, 1999). However, when traits within a trade-off are highly polygenic in their underpinnings, they may be able to evolve outside a simple genotypic trade-off, and therefore, selection on each trait may not result in the same reciprocal response to selection in the other (Stearns & Partridge, 2001). Fundamentally, traits involved in negative genotypic correlations may be tightly linked, but, equally, differences in the genetic architecture of the traits may mean that the effect of selection in the two traits is far from symmetrical (Stearns & Partridge, 2001). Testing whether the impact of selection is reciprocal on many trade-offs is difficult, partly because detecting trade-offs is already challenging (Cressler et al., 2015; McKean et al., 2008); however, it is central to the complete understanding of trade-off relationships (Stearns & Partridge, 2001).

One particularly well-evidenced trade-off is that of the resistance of *Plodia interpunctella* (Hübner) to the baculovirus, 'Plodia interpunctella Granulosis Virus' (PiGV). *Plodia interpunctella*, or the Indian meal moth, is a grain-feeding agricultural pest that is naturally infected by the baculovirus PiGV, an obligate killer that transmits by being orally ingested during larval cannibalism. The larvae of *P. interpunctella* are a widespread grain-feeding pest (Mohandass, Arthur, Zhu, & Throne, 2007), which have been used as experimental study species for its ease of population maintenance and agricultural importance (Mohandass et al., 2007; Silhacek & Miller, 1972). Eggs are laid into cereal media by adults in a semelparous event, larvae then develop in the food media until pupation, and, following pupation, adult moths emerge, mate and lay a new generation of eggs (Gage, 1995). Adults do not have functional feeding physiology; their reproductive success is broadly determined by how quickly they can develop and their pupal mass (Boots & Begon, 1993; Silhacek & Miller, 1972). *Plodia* larvae can be infected by the baculovirus Plodia interpunctella Granulosis Virus (PiGV) (Vail, Hoffmann, & Steven Tebbets, 1993). PiGV infections are obligately lethal following infection via consumption of viral occlusion bodies. Resistance is thought to occur mostly at the gut wall through mechanical barriers such as the peritrophic membrane and apoptosis of infected gut wall cells (Begon, Daud, Young, & Howells, 1993; Tidbury, 2012).

Two notable selection experiments have shown that *Plodia* experiencing selection through exposure to the pathogen evolve resistance to it at the cost of increased development time (Boots, 2011; Boots & Begon, 1993). This trade-off was further confirmed to be both genetic and constitutive (demonstrable even in the absence of exposure to infection), in a recent third experiment through the comparison of inbred lines (Bartlett, Wilfert, & Boots, 2018). As such, this genotypic trade-off is particularly well defined since it has been shown in replicated selection experiments, under different resource conditions, and using inbred lines. This same system has also been used to infer trade-off shape from population level patterns of resistance (Mealor & Boots, 2005), and similar developmental trade-offs in *Plodia* have been demonstrated in the context of bacterial and parasitoid resistance (Niogret, Sait, & Rohani, 2009; Oppert, Hammel, Throne, & Kramer, 2000). However, in all cases, selection has been applied only to resistance and the correlated changes in development time observed (Boots, 2011; Boots & Begon, 1993). We do not therefore know how tight the correlation between the traits is and whether resistance is a constraint on the evolution of development time. Here, we test whether selection in the opposite direction along this trade-off—that is, selection on the development rate, both faster and slower—leads to correlated changes in resistance.

## 2 | METHODS

### 2.1 | Population maintainance and artifcial selection

We maintained *Plodia* populations following well-established protocols. Our selection lines all originated from the same outbred laboratory stock population, which we have shown in previous studies to maintain appreciable amounts of genetic variation in life-history characteristics and resistance to PiGV (Bartlett et al., 2018). We originated eight lines, each as a starting cohort of 60 randomly selected, recently emerged *Plodia* adults (of unknown sex) placed on 200 g of fresh food media inside 1000-ml straight-side wide-mouth Nalgene jars (Thermo Fisher Scientific). We prepared food media in batches consisting of 250 g 'Ready Brek' (Weetabix Ltd.), 150 g wheat bran (Bob's Red Mill), 100 g rice flour (Bob's Red Mill), 100 g brewer's yeast (MP Biomedicals), 125 ml glycerol (VWR), 125 ml clear organic honey (Dutch Gold Honey Inc.), 2.2 g methyl paraben (VWR) and 2.2 g sorbic acid (Spectrum Chemicals). We homogenized

the media with industrial mixers before it was sealed and frozen for a minimum of 24 hr prior to thawing at ambient- temperature for use.

We allowed adult moths to reproduce and then selected 60 of their adult moth offspring to found the next generation in a new jar of food media. How we select these sixty moths is how we differentiate our two selection regimes, dubbed 'early-' or 'late-' selected; of our 8 lines, 4 were assigned to the 'early' treatment, and 4 to the 'late'.

For the early-selected lines, we collected the first sixty next-generation adults that were counted during daily checks to found the next generation. Under this regime, only the very fastest developing larvae (relative to the rest of their population) were allowed to reproduce. For the late-selected lines, populations were checked daily. So long as abundant 5th-instar larvae were present, any adult moths were removed from the population and frozen. Once there were no 5th instar larvae, sixty adults were then randomly selected from the remaining population and transferred to a new jar of food media. In this way, we allowed only slower developing larvae to found the next generation for that line, although we could not guarantee these were the absolutely most slowly developing of their generation. Previous studies in this system have shown no difference in development time (our trait under selection) between male and female moths; for example, Boots and Begon (1994, 1995) show no effect of sex on development time across two large multifactor experiments. Boots and Begon (1993) did find a small significant difference according to sex in their analysis of two populations; if we analyse the data presented (Welch's *t*-test), we find that only one population shows a significant difference on development time based on sex (resistant population: $t_{107.0} = 1.72$, $p = .087$; control population: $t_{97.4} = 2.21$, $p = .029$) with Cohens' d for each equal to $d = 0.32$ and $d = 0.41$ respectively; we point readers to Boots and Begon (1993, 1994, 1995) for development time means and standard errors for male and female moths. We therefore have good evidence that there is broadly no, or only a small, difference in development time between sexes, leading us to believe our selection regime should not have skewed operational sex ratios and altered sexual selection in a meaningful way.

We maintained these selection regimes for approximately four years; however, the number of generations this time period represents is different for each line due to their differences in development time (Table 1). We maintained all selection lines in a single incubator throughout the experiment, where they experienced a constant climate of 27 ± 2°C and 35 ± 5% humidity, with 16:8 hr light:dark cycles. Following this period of maintenance and selection, we removed the selection pressure for two generations of *Plodia* where next-generation founders were randomly selected within each line. This is typical in such *Plodia* studies as ours to try and mitigate plasticity or parental effects (Boots, 2011; Roberts et al., 2019). We then assayed the lines' life history and level of resistance.

## 2.2 | Resistance and life-history assays

We undertook assaying of resistance and development in two blocks: four lines were assayed per block, with two early-selected and two late-selected lines in each block. The two blocks were separated by approximately one calendar month. This protocol was used due to the limitation of asynchronous generation timings between lines.

**TABLE 1** Number of generations of selection experienced by each line at the point of assaying, and which assay block each line was assigned to

| Line | Selection regime | Generations of selection | Assay block |
|---|---|---|---|
| E1 | Early | 52 | A |
| E2 | Early | 55 | A |
| E3 | Early | 48 | B |
| E4 | Early | 51 | B |
| L1 | Late | 37 | A |
| L2 | Late | 38 | B |
| L3 | Late | 40 | B |
| L4 | Late | 37 | A |

We characterized the life-history traits of each line using two measures: time to pupation (development time) and pupal mass. For each line, we took sixty adult *Plodia*, known to have eclosed in the last 24 hr, placed them in jars of new food media and incubated them under the conditions described above. After 11 days, we selected fifty larvae on the 1st day of their 3rd instar from each line and placed them in individual compartments on 25-cell compartmentalized square petri dishes (Thermo Fisher Scientific) (two petri dishes per isoline) with ample food media. We can identify 1st-day 3rd-instar larvae based on the size of their head (which changes only during moulting and identifies different instars) and the size of their body (which if smaller in diameter than the head signifies their 1st day at that instar). Petri dishes were then incubated as above and checked daily to monitor larval development. We recorded the date of each larva's pupation, and two days later, the pupa was extracted from its silk cocoon and weighed using a 1-μg precision microbalance. Growth rate of each individual was calculated as its mass at pupation divided by its days to pupation. Not all larvae were recovered, as some inevitably die due to handling or other causes of stochastic mortality, or are damaged during pupal extraction from cocoons ($\bar{n} = 22.375/50$ pupae recovered per line).

We measured the resistance of each line to PiGV by comparing infection rates of larvae to different PiGV doses. We took 150 1st-day 3rd-instar larvae from each line, following the same protocol as described above. We placed larval cohorts of fifty larvae into circular petri dishes (three cohorts per line) and starved the larvae for one hour. We then pipetted droplets of virus solution into these petri dishes, with each cohort given one of three solutions. Virus solutions represented three doses, each diluted by an order of magnitude (such that the strongest dose is 100 times stronger than the weakest). We diluted solutions with distilled water, and all solutions contained 2% sucrose (Thermo Fisher Scientific) and 0.1% Coomassie Brilliant Blue R-250 dye (Thermo Fisher Scientific). We left larvae to voluntarily feed on the solution droplets, encouraged by their brief starvation and the solution sucrose content. We considered an individual larva

dosed when 50% of their alimentary track was stained blue (visible due to the blue dye and translucent larval body) at which point we removed them from the petri dish and placed them individually into cells of 25-cell compartmentalized square petri dishes, before incubation for twenty days as above. After twenty days, we froze the petri dishes to kill all remaining live larvae, before opening them for counting. Infected larvae are apparent due to their bright white cadavers, a consequence of the accumulation of viral occlusion bodies in the haemolymph. Uninfected larvae were distinguishable as healthy larval cadavers or as developing pupae or adults. Not all larvae were recovered to be categorized as either infected or uninfected, as some inevitably die due to handling or other causes of stochastic mortality ($\overline{n}$ = 16.75/25 larvae recovered per line per dose).

### 2.3 | Additional assays

The eight experimental populations were additionally assayed for both life history and resistance at the beginning of the experiment after the first generation under the selection regime (generation = 1). These assays were all undertaken on different days due to the asynchrony of the experimental populations throughout the selection process and were performed in a different laboratory using a different viral stock solution, and therefore, 'doses' used between the initial generation = 1 assays and the final main assays are not the same. Therefore, the comparison across the different experiments must be interpreted with caution but we include some analyses of these initial assays to better inform the framing of our findings.

Further, following the first analysis of our main findings, we sought to better frame the results of our main experiment in the context of established trade-offs in this system by re-assaying life history and resistance of three inbred populations from Bartlett et al. (2018). To improve comparability of these new assays, we used the same viral stock solutions (same doses) as the main experiment, and the assays we undertaken in the same laboratory in the same year as the main experiment. These assays could not be undertaken concurrently with the assaying of the early- and late-selected lines and so direction comparison is partially confounded by potential day effects.

### 2.4 | Statistical analysis

All analyses were undertaken in R (v.3.4.4—'Someone to Lean On') (R Core Team, 2019).

With the exception of the re-assayed inbred lines, we analysed all assay data using a generalized linear mixed-effects modelling approach to account for our hierarchical experimental design. We tested for significance of fixed effects using the 'afex' package (Singmann, Bolker, Westfall, Aust, & Ben-Shachar, 2019) which integrates with the generalized linear mixed-modelling 'lme4' package (Bates, Maechler, Bolker, & Walker, 2015; Bolker et al., 2009), and coupled this with the 'emmeans' (Lenth, 2019) package to estimate effect sizes where appropriate. For the main experiment, we tested for a significant effect of treatment on growth rate, development time, pupal mass, and susceptibility to the pathogen. Random effects were the blocking factor 'Block' (see Table 1), and 'Line' nested under 'Treatment' to account for our hierarchical experimental structure. 'Treatment' was the only fixed effect for analysis of growth rate, development time, and pupal mass; 'line', 'dose', and an interaction between the two were the fixed effects for the analysis of infection data by line, and 'treatment' and 'dose' were the fixed effects for analysis of infection data by selection regime. Models for growth rate and pupal mass used a Gaussian error structure, for development time used a Poisson error structure, and models for infection assays used a binomial error structure. Because of this, all model comparisons used a likelihood-ratio test ('LRT') method (see documentation for 'afex' package, (Singmann et al., 2019)).

Initial assays of the experimental lines at generation = 1 used the same approach as above, with the exception of not including a blocking factor as each population was assayed on a different day. The three re-assayed inbred lines from Bartlett et al. (2018) were all assayed concurrently and had no hierarchical experimental structure; we calculated simple arithmetic means for their life-history assays, and for infection assays used generalized linear models with a binomial error structure including dose and line as fixed, possibly interacting effects. We examined the rank-order change in line susceptibility from the start to the end of the experiment, according to selection regime, through way of an ANOVA on regular ranks (using the 'npIntFactRep' package (Feys, 2016)).

We further investigated a potential correlation between life-history traits and susceptibility to infection, similar to that shown in Bartlett et al. (2018). We used the GLMMs from our analysis of development time and infection to predict an expected development time and expected proportion of individuals infected at the highest virus dose for all eight lines if they were assayed concurrently. We plotted these values and tested for a correlation using a Pearson's correlation.

We provide an annotated R script and curated data for reproducibility of all analyses in association with this manuscript.

## 3 | RESULTS

### 3.1 | Main experiment

Experimental selection regime had a significant effect on growth rate ($p$ = .03) where the late-selected line growth rate was 0.31 mg/day lower than the early-selected line (Figure 1). This was driven by a significant effect of treatment on development time ($p$ = .02) where late-selected lines pupated 7.0 days later than early-selected lines (Figure 1); consistent with previous work in this system (Bartlett et al., 2018; Boots, 2011; Boots & Begon, 1994), there was no effect of selection on pupal mass ($p$ = .55).

Experimentally selected lines also showed significant variation in their resistance to PiGV (Figure 2). We found no evidence ($p$ = .33) of an interaction effect between 'line' and 'dose', indicating no heterogeneity in terms of dose response (again in

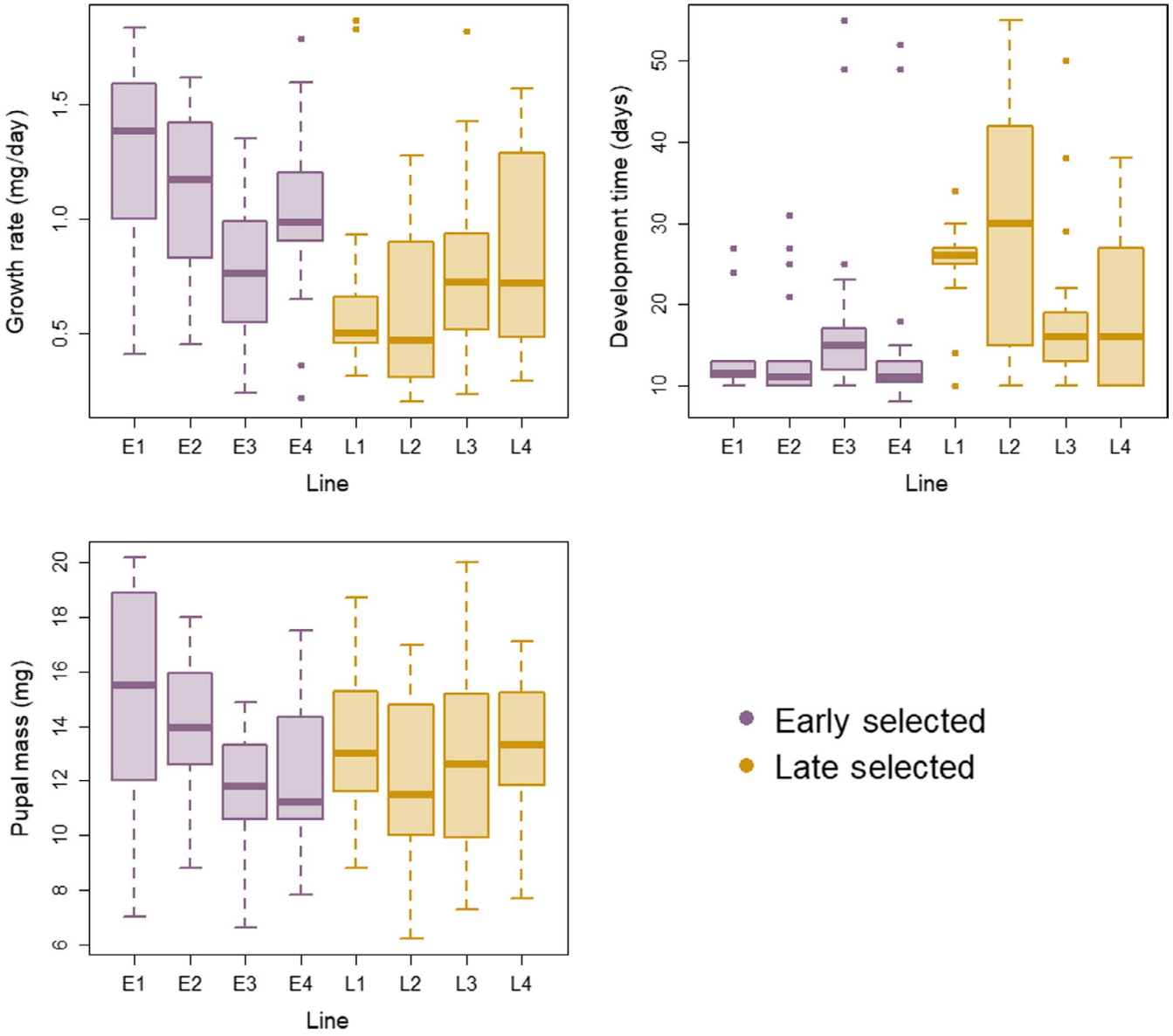


**FIGURE 1** Panelled box plots illustrating life-history traits of each line (growth rate, development time, pupal mass). Early-selected lines are shown in purple and left-aligned on each subplot; late-selected lines are shown in orange and right-aligned on each subplot. Significant differences amongst lines and between treatments were found for growth rate and development time, but not pupal mass. Early-selected lines had shorter development times and therefore higher growth rates

agreement with previous work in this system Bartlett et al., 2018; Boots, 2011; Boots & Begon, 1994)). However, lines did differ in their overall resistance to the pathogen ($p$ = .005), illustrated in Figure 2. We found inconclusive evidence of an effect of selection regime on resistance to the pathogen at the end of the experiment, regardless of if we modelled based simply on 'treatment' (presented here) or based on an estimated cumulative selection differential (identical results, not presented in this study). Late-selected lines were possibly more susceptible to the virus than early-selected lines (Figure 2); estimated effect sizes (likelihood of infection) for early- and late-selection regimes (on a hypothetical average dose) were that early-selected individuals had a 0.09 probability of infection (95% CI: 0.04–0.19) whereas late-selected individuals had a 0.23 probability of infection (95% CI: 0.12–0.39), and the effect of selection regime was not significant ($p$ = .07), although see additional analyses below.

We further investigated the link between line development time and susceptibility to the pathogen by examining if there was a correlation between these two phenotypes across all eight lines (Figure 4). We found no evidence for a correlation between growth rate and susceptibility (Pearson's Correlation, $t_6 = 1.06$, $p = .33$). We do not have the necessary level of replication to test with any power for a correlation between line development time and susceptibility within each treatment, and caution that even for a 'collated' correlation, eight replicate populations remain a low level of replication for even simple correlative analysis.

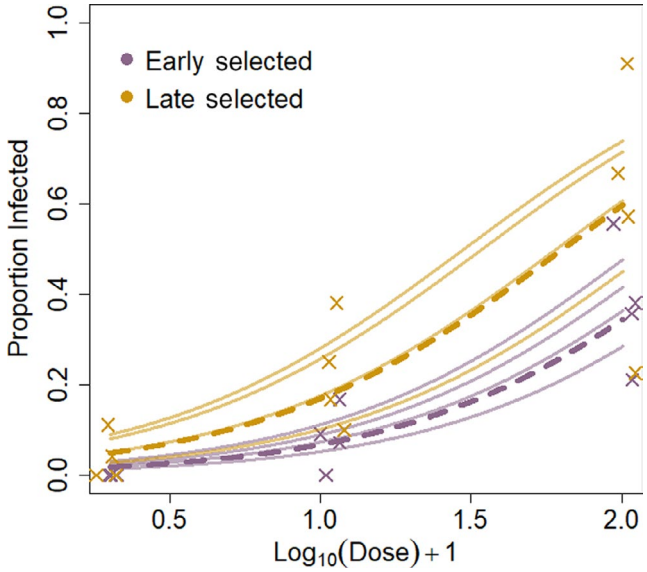


**FIGURE 2** Plot showing resistance of each line to PiGV. Plotted crosses are proportion of larvae infected, where dose strength is plotted in logit space (note that a jitter has been applied to the plotted points along the *x*-axis for easier visualization). Plotted solid curves represent the expected proportion of infected larvae with increasing dose for each line, and the heavier dashed curves represent the predicted proportions infected based off treatment; all curves are predicted values from the corresponding GLMMs. The early-selected lines are plotted in purple; the late-selected lines are plotted in orange. Lines do not show heterogeneity of dose response, but do significantly differ in their resistance to PiGV

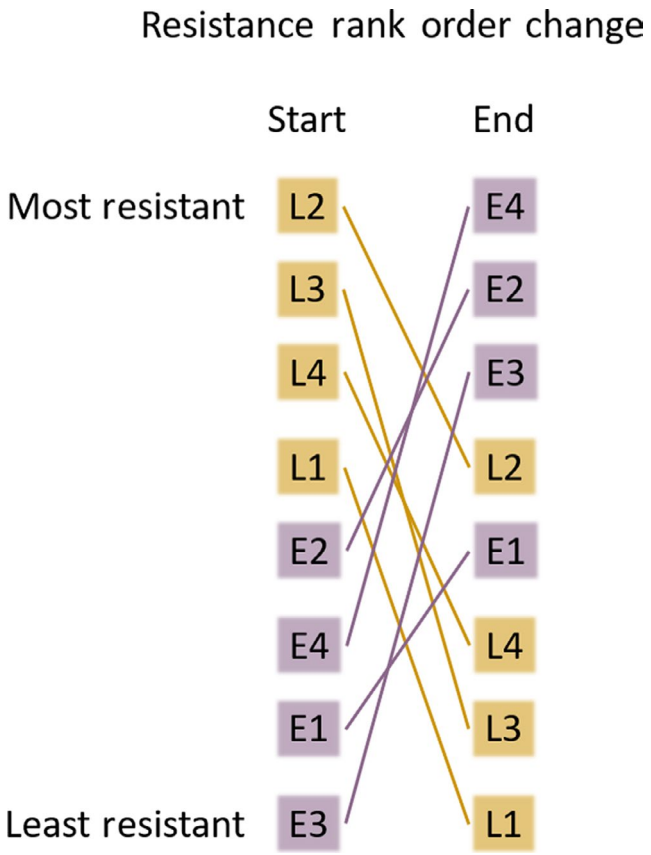


**FIGURE 3** Rank-order change in resistance to PiGV of replicate *Plodia* lines at the start (after one generation) and end (after ≥37 generations, see Table 1) of the selection experiment. We find a significant effect of selection regime on the rank-order change between the start and end of the experiment (ANOVA on regular ranks: $F_{1,6} = 18.3$, $p = .005$). We present evidence (confounded by different assay dates) that there was a stochastic bias at the start of experiment, where late-selection lines were significantly more resistant ($p = .005$)

### 3.2 | Additional assays

We analysed two additional data sets following our main analysis, to better frame the results of our main experiment. We undertook a similar analysis as above on assays of life history and resistance of the 8 experimental lines at generation = 1 (after one single generation of selection). These analyses are confounded by each line being assayed on a separate day, as during the selection experiment the populations are asynchronous in their reproductive bouts, and so results should be interpreted cautiously. We found no significant difference in growth rate at the treatment level between the lines set-up to be late-selected or early-selected ($p = .93$), illustrated in Figure S1 (Supplementary Material). We did find evidence of a bias in resistance at set-up between the two treatment groups ($p = .005$), however this was in the direction of the late-selection populations being more resistant than the early-selection population—opposite to the direction of the difference in susceptibility according to treatment at the end of the experiment. We cannot directly, quantitatively compare start and end of experiment population susceptibilities as they were assayed using different viral stock; however, we can examine the rank-order change of line resistances across selection regimes between the start and end of the selection experiment (see Figure 3). A regular-ranks ANOVA shows a significant effect of selection regime on the change in line resistance rank order from the start to the end of the experiment ($F_{1,6} = 18.3$, $p = .005$), where early-selected lines were significantly more likely to move up the resistance rank order and late-selected more likely to move down. This suggests that during the experiment, late-selected lines became more susceptible, early-selected lines became less susceptible, or both.

For comparison to our experiment end-point results, we analysed the susceptibility to infection and growth rates of our three re-assayed inbred lines surviving from Bartlett et al. (2018), calculating a mean development time and estimated likelihood of infection at the highest dose; notably for these populations, assays were undertaken shortly after the assay of our main selection experiment using the same conditions and same virus stock, however were not assayed on the same days as our early and late lines. We present a replotting of Figure 4 in the Supplementary Material (Figure S2) showing how these inbred lines compare to the selection lines in susceptibility/ development time phenotype space.

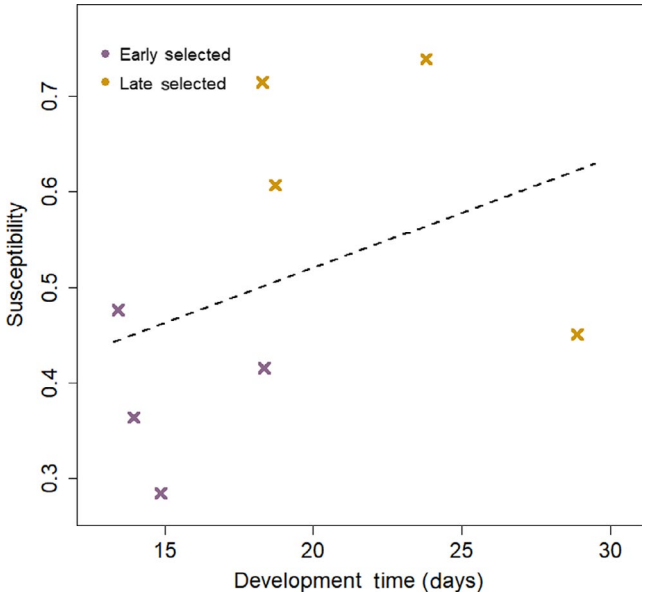


**FIGURE 4** Graph showing expected likelihood of infection at max dose (susceptibility) and expected development time of each line, based on model predictions. Although the general finding that early-selected lines (purple) were significantly faster growing and possibly less susceptible than late-selected lines (orange) is apparent, there was no strong compelling evidence for a correlation between growth rate and susceptibility on a line-by-line basis

## 4 | DISCUSSION

Our results emphasize the importance of examining the fundamental underpinnings of both sides of genotypic trade-offs, as results from selecting on one trait may not be reflected when selection acts on the other. We successfully selected on development time (Figure 1), and consequently overall growth rate, with no change to pupal mass. Our finding of no significant effect on pupal mass is in agreement with previous work selecting on resistance (Bartlett et al., 2018; Boots, 2011; Boots & Begon, 1993; Oppert et al., 2000), but is notable in that here we were directly selecting on a life-history trait. Our key result is that early-selected lines were equally or less susceptible to infection (Figures 2 and 3) compared to late-selected lines, counter to the well documented trade-off between pathogen resistance and development time in this species found when we select on resistance (Bartlett et al., 2018; Boots, 2011; Boots & Begon, 1993; Oppert et al., 2000). That is resistance and development time are negatively correlated when we select on resistance or when populations evolve by genetic drift, but are unlinked or possibly positively correlated when we select on development time. Our results should not be interpreted as evidence against the existence of an otherwise robustly supported genotypic trade-off between resistance and development time in our system. Rather, our findings offer some insight into the likely polygenic underpinnings of these traits, the mechanisms of immunity in this system, as well the potential caveats of the interpretation of selection experiments.

Our key result is that when we selected on development time, we found evidence of a correlated response on resistance that is inconsistent with, or even opposite to, the established trade-off in this system. The previously documented trade-off is constitutive and genetic (Bartlett et al., 2018), but we have no explicit data on how many genes may be involved in resistance and linked to development time. Ongoing work in this system putatively points to multiple, context-dependent evolutionary routes to resistance (Roberts et al., 2019), and that resistance traits are polygenic. It is clear that variation in insect life-history phenotypes are also often highly polygenic (Comeault et al., 2014; Jha et al., 2015), with many interacting genes responsible for determining fecundity, growth rates, or size at maturation. Our results are likely to be a consequence of such polygenic underpinnings where the set of genes that responded to selection on development time here were not those that affect resistance in the predicted direction. This shows that the full set of genes governing life-history phenotypes have mixed correlations with those involved in viral defence. An informative ideal test of these observations in this system would be a single experiment where populations are subjected to a $2 \times 2$ selection regime, combining early versus late selection on development time (as here) and exposure or no-exposure to PiGV (as in previous selection experiments). Such a selection experiment would illustrate the possible multiple routes to immunity which may involve in different contexts, as has been recently done in this system comparing nutritional selection regimes in tandem with virus exposure regimes (Roberts et al., 2019).

Similar experiments selecting along both traits in a trade-off have been conducted in the life-history experimental evolution literature (summarized in Stearns and Partridge (2001)). For example, Hillesheim and Stearns (1991) and Zwaan, Bijlsma, and Hoekstra (1995) both examined the life-history trade-off between weight at eclosion and development time. Hillsheim and Stearns (1991) selected on body size, whereas Zwaan et al. (1995) selected on development time. In this case, the trait under selection did not affect the correlation of these traits—in both experiments larger flies had longer development time. In a second analysis, Hillesheim and Stearns (1992) examined the lifespan of fly lines selected for divergence in body size and found that larger flies had shorter lives than smaller ones. However, when Partridge, Prowse, and Pignatelli (1999) selected for longevity, they found no difference in weight between long and short lived flies. Additionally, when Stearns, Ackermann, Doebeli, and Kaiser (2000) selected for increased intrinsic mortality by increasing extrinsic mortality rates, they found that flies were both shorter lived and smaller. In these cases, the sign of the genetic correlation of these traits in this system varied depending on the trait under selection as it did in our experiment here in comparison to previous experiments elsewhere.

As molecular genetic and sequencing methods become increasingly widespread and affordable, efforts to link specific genes to selection experiments or evolutionary trade-offs have become more common (Korte & Farlow, 2013). However, identifying the genetic bases of trade-offs remains challenging, especially when traits are determined by more than a few pleiotropic or linked genes. There are systems where there has been success in identifying pathogen-resistant quantitative trait loci (Zhong, Pai, & Yan, 2005); however,

our findings suggest that it will be challenging for current genomics approaches to determine the full set of genes that co-regulate the resistance-development time trade-off in *Plodia* (Gassmann, Onstad, & Pittendrigh, 2009). Our selection experiment emphasizes that the set of genes identified in quantitative trait locus studies will depend on the population's selective history and therefore that these results from experimental evolution should be interpreted cautiously. However, reciprocal selection experiments such as ours could be harnessed to identify more complete sets of pleiotropic genes since selection experiments along multiple axes may select on a broader set of pleiotropic genes that underlie linked traits.

The differences in resistance that we observed have multiple plausible explanations with potential insight for related future experiments. One possible hypothesis is that haemocoelic immunity, which has been shown to vary between populations in this system (Saejeng, Siva-Jothy, & Boots, 2011), is unaltered between our selection lines, whereas midgut immunity is inherently greater in the fast-developing lines due to greater likelihood of shedding viral occlusions bodies before infection occurs through accelerated ecdysis. Engelhard and Volkman (1995) showed in a similar lepidoptera-baculovirus system that an age difference between larval instars of just a few hours significantly affects infection likelihood and that larvae were able to fully clear early infections from the midgut epithelium during ecdysis. Interestingly, similar work in mosquitoes has shown that faster development correlates with increased resistance to an ingested pathogen (Koella & Agnew, 1999; Yan et al., 1997), yet some of those same authors show that late-selected mosquito lines exhibit higher haemocoelic immune activity than early-selected lines (Koella & Boëte, 2002), counter to their previous findings. Furthermore, there is fundamental theoretical work exploring how accelerated development can be an adaptive response to age-structured infection (Hochberg, Michalakis, & Meeus, 1992). It is therefore plausible that this study has indirectly increased or maintained resistance in the faster-developing lines by allowing larvae to escape the establishment of successful infection by developing quickly, potentially through more rapid ecdysis after inoculation. Although there are numerous insect-resistance studies that similarly seem to identify such 'costless resistance' (Faria et al., 2015; Milks, Myers, & Leptich, 2002; Undorf-Spahn et al., 2012), these findings are difficult to reconcile with theory and widespread observation in variation of resistance (Koskella, 2018; Schmid-Hempel, 2003; Susi & Laine, 2015), and it is acknowledged that trade-offs are difficult to tractably characterize experimentally (Cressler et al., 2015). If our early-selected lines have evolved to develop faster without a loss of, or even with a corresponding gain in resistance, we speculate it is at the expense of some other competitive axis which doesn't manifest in these experiments.

It must also be emphasized that the early-selected lines may not be benefiting from higher resistance, but that the nature of our experimental design may have led to late-selected lines being particularly vulnerable to infection. It is well established that many mutations are purely detrimental, constituting 'genetic load' (Crnokrak & Barrett, 2002; Whitlock & Bourguet, 2000; Wielgoss et al., 2013); captive managed (laboratory) populations may harbour much larger genetic loads than their wild counterparts (Bryant & Reed, 1999) on the basis of relaxed selection. Reduced growth rate is a disadvantageous trait (Boots & Begon, 1993; Bowers, Boots, & Begon, 1994; Silhacek & Miller, 1972), and therefore by selecting for a broadly less-fit phenotype, we may have simply inadvertently selected for individuals of broadly low quality that harbour large numbers of deleterious alleles and significantly elevated genetic loads across the population. As such, our 'late-selected' treatment may have manifested as selection for poor performing low-fitness phenotypes, and therefore it may be no surprise that these populations show elevated susceptibility to infection. Without characterizing the ancestral phenotypes at the same time as our two selection lines, we cannot determine if both our early-selected and late-selected lines show changed susceptibility and development time compared to their ancestral state. We have data on the ancestral case demonstrating changes in relative development times and susceptibility, but without assaying a non-selected 'ancestral' treatment alongside the early- and late-selection treatments, we cannot make informative direct comparisons.

Future investigations of these selection lines may provide insight if assayed in direct comparison to a larger number of inbred lines similar to those described in Bartlett et al. (2018) under one single experiment. We partially attempted this using the three surviving inbred lines from the Bartlett et al. (2018) study; however, note that those lines were assayed on a separate date to blocks A and B of this main experiment (Table 1) and so direct comparisons are difficult. However, we still present a tentative appraisal of how our selected lines compare to these inbred lines in Figure S2 (Supplementary Material); we note that the inbred lines from Bartlett et al. (2018) were originated from the same outbred starting population at approximately the same as the selection lines presented in this study. Although our interpretation is speculative, the apparent result is that the early-selected lines mostly sit along the same trade-off as the three assayed inbred lines, whereas the late-selected lines have moved away into phenotype space which would under a wild-type scenario be seen as noncompetitive. This suggests that the late-selected lines have not evolved along the trade-off, but may rather have evolved away from it by increasing development time with either no change, or a decrease, in resistance to the virus. This could be through accumulation of broadly deleterious mutations, or a demonstration of the highly polygenic nature of life-history traits, as discussed above.

In conclusion, our results suggest a polygenic underpinning of the established trade-off in *Plodia* and its immunity to PiGV. These results highlight potential mechanisms of immunity which may be worth further investigation, notably accelerated growth to escape infection, and in this case rapid ecdysis clearing midgut epithelial infection. Finally, this study illustrates some of the challenges of selection experiments investigating trade-offs, such as the potential of inadvertently selecting for evolution away from, rather than along, a phenotypic trade-off, especially by accumulation of broadly deleterious mutations.

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## AUTHOR CONTRIBUTIONS

K.R. and M.B. designed the study; L.J.B., KR. and E.V. maintained the experiment and gathered data with Y.H.; E.V. and Y.H. curated the data; L.J.B. analysed the data with guidance from M.B.; and L.J.B. and E.V. interpreted results and drafted the work, with major contributions from all authors to the first and all subsequent drafts.

## DATA AVAILABILITY STATEMENT

All data presented in this study and its associated analysis script are made available in association with this manuscript as a Dryad Data set; Citation: Bartlett, Lewis et al. (2020), The target of selection matters: An established resistance—development-time negative genetic trade-off is not found when selecting on development time. Dryad, Dataset, https://doi.org/10.5061/dryad.7sqv9s4q3.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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