



DEPARTMENT OF BIOLOGICAL AND  
ENVIRONMENTAL SCIENCES

# HOW ENVIRONMENTAL VARIATION AFFECTS GENETIC TRADE-OFFS AMONG LIFE-HISTORY TRAITS

**Deborah van Putten**

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Supervisor: Luc Bussière Department of Biological and Environmental Sciences

Examiner: Mats Olsson Department of Biological and Environmental Sciences



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## Abstract

To predict how populations will adapt and evolve, it is essential to understand how costly life-history traits genetically covary and trade off in response to variable environments. These trade-offs can limit the response to selection and thus can constrain adaptation in populations. Understanding these genetic trade-offs is especially important for managing pesticide resistance in pest populations.

My thesis aims to quantify genetic effects between life-history traits and varying environmental conditions, in *Helicoverpa armigera* moth larvae infected by different doses of a fungal biopesticide. Survival, pupal mass and development rate were measured as response variables. To estimate genetic effects, a half-sibling design was used to partition sire variance from the phenotypic variation. Although the biopesticide did not appear to affect traits on a phenotypic level, including low mortality across all doses, the Bayesian model revealed some evidence for genetic effects.

Genetic correlations within development rate were highest for similar doses and lowest for vastly different doses, suggesting gene-by-environment interactions. A positive genetic correlation between pupal mass and development rate was found in the highest dose, while the genetic correlations in the lower doses were closer to 0. This may suggest that certain genetic effects only influence performance under high stress conditions, and that genetic correlations can be masked at low stress environments, due to favorable conditions.

Despite limited statistical power due to the number of half-sibling families, my results show that genetic effects are environment-dependent. My thesis shows the importance of considering environmental variability when predicting evolutionary responses, especially in the context of managing pesticide resistance, where hidden genetic constraints may only emerge under stress.

**Keywords:** *Gene-by-environment interactions, life-history traits, trade-offs, resource acquisition, evolutionary constraints, genetic variation*

## 1.0 Introduction

In evolutionary biology, understanding how life-history traits covary genetically gives important insights into the constraints of evolution and adaptation (Garland et al., 2021; Roff & Mousseau, 1987; Via, 1987). Life history traits often trade off, meaning that an advantage in one trait leads to a disadvantage in another trait, limiting the potential simultaneous improvement in both traits (Cohen et al., 2019; Stearns, 1989). However, the strength and direction of these trade-offs are not fixed; instead, they can vary depending on the environment (Via & Lande, 1985). Trade-offs can arise from various mechanisms, including physical and biochemical conflicts, limitations in resources, and genetic mechanisms like antagonistic pleiotropy and linkage disequilibrium (Agrawal, 2019; Garland et al., 2021; Jessup & Bohannan, 2008). When these diverse mechanisms interact with fluctuating environmental conditions, they can cause trade-offs and influence their direction, adding to the complexity of evolutionary adaptations via gene-by-environment (hereafter GxE) interactions (Stearns, 1989).

GxE interactions are evolutionary mechanisms in which gene expression differs depending on the environment. Depending on how expression covaries with fitness, GxE can sometimes lead to genetic trade-offs within or between life-history traits, such as growth, reproduction, and immune defense. (Blanford et al., 2002; Lazzaro & Little, 2008). Indeed, in natural systems, populations exhibit within-population genetic variation for life-history traits important for survival and growth (Lazzaro & Little, 2008; Tinsley et al., 2006). In natural systems, selective pressures are highly dynamic and influenced by environmental changes such as temperature, food availability, and pathogens, and these fluctuating environmental pressures contribute to the maintenance of genetic variation in these populations (Lazzaro & Little, 2008). Understanding how genetic variation and GxE interactions can shape trade-offs between or within traits is important for understanding for evolution and adaptation of populations.

A negative covariance between phenotypic trait values (for instance, high reproduction and low body weight) could potentially indicate a genetic trade-off, based on models in which organisms must allocate limited resources between costly traits (Haave-Audet et al., 2021; Stearns, 1989). Yet multiple studies have found positive phenotypic covariances (for instance, high reproduction and high body weight) when genetic trade-offs were expected (Haave-Audet et al., 2021; Moiron et al., 2019; Royauté et al., 2018). The presence of a positive covariance does not rule out the possibility of a genetic trade-off. As Van Noordwijk and De Jong (1986) argue, the ability of an organism to acquire resources (i.e. food), known as resource acquisition, can mask such genetic trade-offs. One way to think about this is with the 'big house, big car' analogy: In a world where everybody has the same amount of money, individuals face a trade-off: they can buy a big house or a big car, but not both (Reznick et al., 2000). This leads to a negative covariance between car and house size. However, in reality, people differ in the amount of money they can spend. Individuals with more money can afford both a big house and a big car, which can lead to a positive covariance between house and car size. Despite the observation that people with nice houses tend also to have expensive cars, there is still a fundamental trade-off, because individuals with more resources cannot spend the same resources on both the big house and the big car. Nevertheless, this trade-off is less apparent among individuals than it would be if one could control differences in resource acquisition (Reznick et al., 2000). This same logic also applies in biology: an organism able to acquire more resources can also allocate more resources to several phenotypic traits (i.e. growth and immune defense), leading to a positive phenotypic covariance, and masking a potential underlying genetic trade-off. For example, King et al. (2010) confirmed this experimentally by manipulating food availability in *Gryllus firmus* to demonstrate that variation in resource acquisition can indeed lead to positive correlations between life-history traits.

The amount of resource acquisition between individuals can vary due to several mechanisms such as genetic differences among individuals and environmental factors such as resource availability or fluctuations in environmental conditions (Van Noordwijk & De Jong, 1986). Additionally, genotypes that perform well in acquiring resources in one environment, might not do so in another environment, meaning that resource acquisition can also be influenced by GxE interactions (Reznick et al., 2000). This variability in resource acquisition and allocation strategies suggests that genetic covariances between life history traits in

a population can shift depending on the environment, due to GxE interactions that can change how selection operates on genetic variation.

Estimating the genetic correlations (or covariances) is useful for discovering genetic trade-offs that may be masked on a phenotypical level by variation in resource acquisition (Reznick et al., 2000; Van Noordwijk & De Jong, 1986). Genetic correlations explain how two traits covary depending on shared genetic factors. For example, a positive genetic correlation between two costly traits means that alleles increasing one trait also tend to increase the other, or that related individuals tend to perform well in both traits. Negative correlations on the other hand indicate that alleles increasing one trait tend to decrease the other, indicating a genetic trade-off. A positive genetic correlation involving two expensive traits (just like houses and cars) suggests that genetic variation for resource acquisition is the main influence on phenotypic trait expression, rather than genetic variation in resource allocation. Therefore, exploring how the genetic correlations between traits change across different environments, can tell us something about how resource allocation and acquisition is affecting the phenotypic trait.

In my research, I will focus on three costly and important life history traits: immunity, pupal mass, and developmental rate. In evolutionary biology, immunity is often regarded as a key fitness-related trait, especially in the study of host-pathogen interactions, which describe the evolutionary arms race between a host and pathogen as they adapt in response to each other (Sheldon & Verhulst, 1996; Sironi et al., 2015). Immunity is such an important trait because survival is necessary in order to reproduce and contribute genes to the next generation, which is central to biological fitness. However, immunity can also be very costly, as the immune system demands energy and resources that could otherwise have been used for other fitness-related traits (Kraaijeveld et al., 2002; Lochmiller & Deerenberg, 2000). As a result, organisms need to balance resource allocation between immunity and other important fitness-related traits in order to increase fitness. Such a balance is often achieved through plastic allocation to immunity: the expression of immune traits is increased in the presence of a pathogenic challenge.

Two important life-history traits that are costly, linked to fitness, and might trade-off with immune investment are development rate and growth. Growth is linked to resource acquisition and is often linked to reproductive potential (Armbruster & Hutchinson, 2002; Honěk & Honek, 1993; Kasamatsu & Abe, 2015; Steinwascher, 1982). Development rate is an important life-history trait as it affects time of exposure to potential natural enemies and the timing of reproduction (Sibly & Calow, 1986). A high development rate can reduce the time available for resource acquisition, which can impact the allocation of resources towards growth (Nylin & Gotthard, 1998). These two traits are interesting to study in relation to each other, since a positive genetic correlation between development rate and growth can indicate genetic variation in resource acquisition: individuals that can grow fast and large at the same time are obviously able to acquire more resources. A negative genetic correlation means that individuals might grow big but need a long development time to do so, or vice versa. This correlation would indicate that genetic variation in resource acquisition is exerting less influence on the life-history traits. Previous studies have looked into the relationship between development rate and growth before, but the sensitivity of this association is not yet clear under varying immune challenges, which could exert pressure on the trade-off because of costly plastic immune investment. Understanding how genetic correlations between these traits shift across environments can therefore help us understand how resource acquisition and allocation can potentially constrain adaptation.

The general study of adaptation is relevant both for pure and applied scientific reasons. As the global population is expected to rise to 10 billion by the 2080s, ensuring food security has become increasingly more important (United Nations, 2024; Carthy et al., 2018). One of the major challenges of feeding the growing populations is mitigating crop loss due to pests (Oerke & Dehne, 2003), and understanding pest dynamics is crucial in this context. Human intervention in agricultural pest control has affected the evolution of traits like pest resistance. Traditionally, synthetic pesticides have been widely used to protect crops. However, the widespread use of these synthetic pesticides has led to a rapid evolution of pesticide resistance in pest populations, reducing their long-term effectiveness (Ayilara et al., 2023). The genetic basis underlying the development of resistance to synthetic pesticides usually only involves a few genes, allowing pests to adapt

more easily and faster, accelerating the spread of resistance in pest populations, and undermining pest control strategies (Ffrench-Constant, 2013).

In recent years, more research has been conducted on using biopesticides (natural organisms or substances derived from them) as an alternative and more sustainable pest control strategy in agricultural practices (Ayilara et al., 2023). Biopesticides interact in a more complex way with the pest immune system than synthetic pesticides by often targeting more physiological pathways (Liu et al., 2019). This complexity gives an advantage because resistance developing in pests is usually polygenic. As a result, more genetic changes are needed for pests to develop resistance, making it a potential solution to resistance development (Tinsley et al., 2006). One potential strategy to undermine pest resistance development and maintain genetic diversity is employing GxE interactions when using biopesticides (Bürger & Gimelfarb, 2002). By changing environmental pressures, farmers can prevent directional selection for a single optimal phenotype. A phenotype selected for in one environmental condition might not be selected for in another environmental condition.

However, modern agricultural practices have become increasingly homogenous with large-scale monocultures (Wuest et al., 2021), creating an environment where pests are exposed to the same selective pressure over time. Previous research has already found promising results that diet-induced genetic trade-offs affect pest survival (Mangan et al., 2025). While it is essential to find practical ways for farmers to incorporate environmental variation, farmers may not need to make significant adjustments. The spraying of the biopesticide itself can already introduce natural variations in doses creating diverse selection pressures, as not every pest will get the same dose level. Ecological theory suggests that this type of environmental variation, similar to the use of refuges in host-pathogen interactions, can create unstable population dynamics and change the direction of evolution, which is important for the long-term effectiveness of the biopesticide (Holt et al., 1999). There is already evidence that different doses lead to different outcomes on life history traits. Higher doses generally lead to higher mortality, but low doses like sublethal doses have been shown to lead to reduced fitness, including lower reproductive success and decreased food consumption (Wakil et al., 2022). These patterns suggest potential trade-offs, as the allocation of resources to survival seemingly compromises other fitness-related traits. However, to the best of my knowledge, research has yet to be done on whether varying biopesticide doses can cause genetic trade-offs between and within fitness-related traits.

To investigate potential genetic trade-offs, this thesis will use the cotton bollworm (Lepidoptera: Noctuidae: *Helicoverpa armigera*) as a model species. The cotton bollworm is a major pest in South America and has a well-documented history of developing resistance to pest control measures, making it a good model species for studying these evolutionary dynamics (Haile et al., 2020). This research focuses on three life-history traits: immune defense, pupal growth, and development rate. Immune defense will be measured as the survival after infection. Growth will be measured as the pupal mass after the larva pupates. Pupal mass reflects resource acquisition during development, but more importantly, it is an indicator of adult fitness, since the resources acquired during the larval stage are vital for reproductive success (Armbruster & Hutchinson, 2002; Steinwascher, 1982). This study also focuses on the development rate, defined as how quickly larvae progress from egg hatching to pupation. Development rate is an important life history trait as it affects the exposure to natural enemies and generation time. However, a high development rate can reduce larval feeding time and therefore decrease access to resources which can lower pupal mass. Therefore, although both a high pupal mass and development rate should be favored under natural selection, we expect that they often trade off (Nylin & Gotthard, 1998). Furthermore, immune defense is a costly life-history trait, and will require more resources the more the pathogen dose increases (Moret & Schmid-Hempel, 2000; Zuk & Stoehr, 2002). Seeing how the genetic correlation between pupal mass and development rate changes across doses can give insight into resource allocation and acquisition patterns. Understanding how different environments influence the genetic effects on these traits will help better understand the evolution and adaptation of pest populations.

## 2.0 Aim

My thesis aims to quantify the genetic parameters, including covariances and variances, for important life-history traits, like immune defense, pupal mass, and development rate in response to different biopesticide doses in the cotton bollworm (*H. armigera*). Specifically, I ask:

- (1) Do within-trait genetic correlations across doses indicate the presence of gene-by-environment (GxE) interactions?

I hypothesize that the more similar the dose difference is, the higher the within-trait genetic correlations across doses are, as I expect genes contributing to trait values at these doses to be similar. In contrast, I expect the genetic correlations to be lower for big dose differences, as I expect high-performance genes in one context to be disadvantageous or irrelevant for trait values in very different contexts.

- (2) Is genetic variation in life-history trait expression mainly affected by resource acquisition or allocation?

If most genetic variation is due to differences in resource acquisition, I expect to see mostly positive genetic correlations among traits at the same dose. This is because higher-performance individuals have a bigger budget to invest in multiple traits at the same time (big house and big car). In contrast, if most genetic variation is due to differences in resource allocation, I expect to see more negative genetic correlations among traits at the same dose. This is because high individual performance in one trait will come at a genetic cost to investment in another trait (either big house or big car).

## 3.0 Material and methods

### 3.1 Experimental system

#### 3.1.1 Moths

The moths used for mating were ordered as pupae from Andermatt Biocontrol AG (Switzerland) in two shipments one week apart. From the first shipment, only male pupae were kept and held at 27°C. From the second shipment, only female pupae were kept and held at 25°C. This separation was done to prevent sibling mating and to synchronize reproductive maturity. All pupae were placed in climate chambers (S600, Aralab, Portugal) under reversed photoperiod conditions (14h light:10h dark, with the dark period between 3:00 and 13:00) and at 75% relative humidity. After eclosion, adults were transferred to *Drosophila* vials with a cotton ball with adult food (Armes et al., 1992). All adult moths were kept under the following conditions: 14h light at 25°C, 10h dark at 16°C, with the dark period between 3:00 and 13:00, and at 75% relative humidity.

#### 3.1.2 Biopesticide

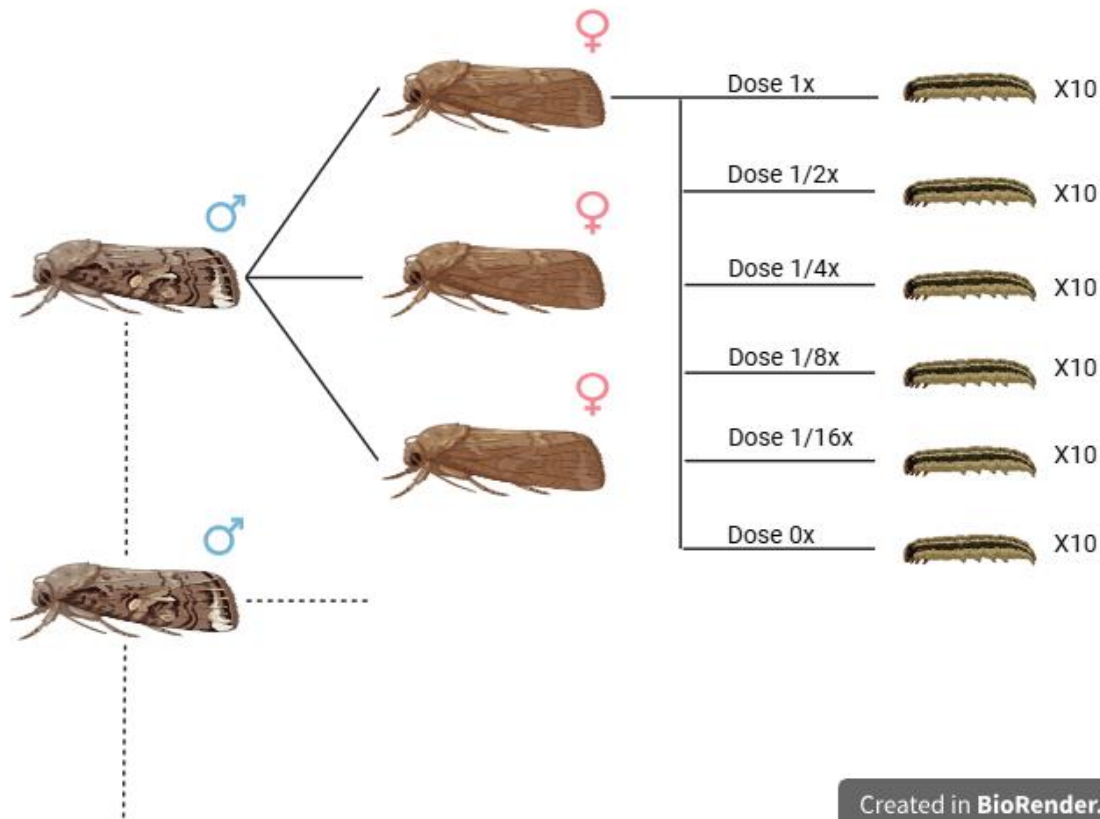
The biopesticide used in the experiment was NoFly, a spore-based insecticide derived from the ascomycote fungus *Cordyceps fumosorosea* (Hypocreales: Cordycipitaceae), produced by Futureco Bioscience. The solutions were made on the day of the inoculation, following the manufacturer's instructions. The doses were made by doing two-fold serial dilutions from the original solution.

### 3.2 Experimental design

#### 3.2.1 Mating set-up

To quantify genetic markers on the different measured traits, we used a half-sibling family experimental design, by mating sires with up to 3 virgin dams, see figure 1. Upon mating, the sires were often a few days old, while the dams were 1 to 2 days old. The sire and dam were put together in a mating box in a dark room for about 30 minutes. Matings were considered successful if the pair stuck together for 20 minutes or longer. The males were then removed and kept for further matings with a recovery period of one day.

Females were placed in an eggpot and food until egg laying. If a mating was unsuccessful, the dam was removed and another was added.



Created in BioRender.com 

**Figure 1: Mating set-up and treatment groups.** One sire is mated with up to three dams. Sixty offspring of each dam are evenly divided into six treatment groups. Figure created with <https://BioRender.com>.

### 3.2.2 Larvae handling and treatment groups

Upon hatching, larvae were fed an artificial diet prepared in the laboratory. On the second instar, larvae were randomly assigned to a treatment group using a random number generator. Each treatment group contains 10 larvae per dam, with up to 60 larvae per dam in total (see figure 1). Larvae were transferred to a clean petri dish with a brush, and 0.5 microliter of their corresponding dose was pipetted directly on top of the larvae before they were transferred to an individual vial with artificial food (larvae of this species need to be individually housed to avoid cannibalism). The brush was cleaned with 70% ethanol in between treatments to avoid cross-contamination.

Larvae were then monitored daily for mortality up to day 14 after inoculation. After day 14, larvae were checked daily for pupation, and pupation dates were recorded for development rate. The pupae were then cleaned of debris with a dry brush and weighed and sexed on the same day of pupation.

### 3.3 Statistical analysis

All statistical analyses were performed in R version 4.4.1 (R Core Team, 2024). To estimate the effects of different doses of fungal spores, I computed the mortality as the proportion of larvae that died between day 3 and 14 divided by the total number of larvae that survived past day 3. I excluded deaths before day 3 to avoid mortality due to handling errors and not from the pesticide.

To avoid confusion arising from sex-dependent differences in development rate and pupal mass (Chen et al., 2014), I focused development rate and pupal mass analyses on males only. In order to

make biological interpretability between the pupal mass trait and the development rate more intuitive I calculated development rate as one divided by the number of days to pupation (higher values representing faster development).

I estimated the genetic parameters by building a multivariate multilevel model with pupal mass and development rate serving as response variables (McElreath, 2020a). Bayesian modeling is ideal for handling multilevel structures, such as those in my experimental design, and for estimating quantitative genetic parameters, e.g., using half-sibling families to estimate genetic effects. To compare the two traits with different units, I log-transformed and standardized both the development rate and pupal mass. The model included dose as a fixed effect and sire and dam as random effects. Only the sire effects were allowed to vary by dose, whereas the dam effect was modeled as a single random effect across doses. The reasoning behind this is that the additive sire effect is considered purely genetic, while the additive dam effect includes both genetic and maternal effects (e.g. egg provisioning), the latter of which is assumed to affect offspring similarly across doses. The mathematical notation for the full model including priors is represented in appendix 2.

I estimated posterior distributions for all parameters using Markov Chain Monte Carlo (MCMC), using the Ulam function from the rethinking R package (McElreath, 2020b). The ulam function uses specifically Hamiltonian Monte Carlo (HMC) sampling, as implemented in Stan version 2.32.7 (Stan Development Team, 2024). I validated the priors in the model using prior predictive simulations, comparing simulated data from the priors with the observed data to ensure that the model's assumptions were reasonable. Furthermore, I performed diagnostics to assess that the model worked as intended. After running the model, all four chains ran successfully without any divergence. Both the trunk and trace plots indicated that the chains mixed well. The R-hat values were close to 1.00 (max 1.01) indicating that the HMC chains have converged successfully. The effective sample size (ESS) values were generally large, suggesting that the HMC sampling was effective.

Heritability estimates were calculated for each trait in each dose using the sire, dam, and residual variances. Specifically, I used the sire, dam, and residual standard deviations extracted from the model to calculate narrow-sense heritability. Narrow-sense heritability is calculated as the proportion of additive variance to the total phenotypic variance (Falconer, 1989). Half-sibling families are expected to share on average a quarter of their additive genetic variances (Falconer, 1989). The total phenotypic variance contains both the additive variance from the sire, the dam variance, and the residual variance. The heritability formula is as follows:

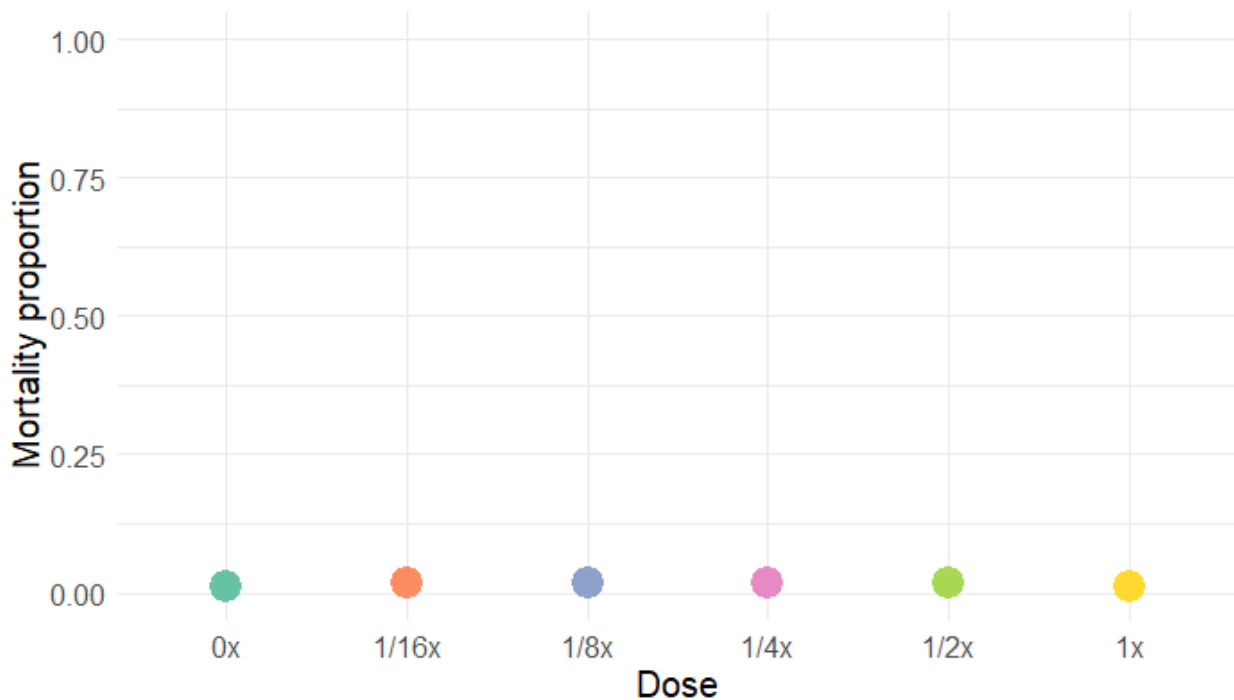
$$h^2 = \frac{4 \cdot \sigma_{\text{sire}}^2}{4 \cdot \sigma_{\text{sire}}^2 + \sigma_{\text{dam}}^2 + \sigma_{\text{residual}}^2}$$

To estimate for within and between trait and dose trade-offs, I constructed a genetic variance-covariance matrix (G-matrix). My matrix provides posterior mean estimates of the genetic variance, covariance as well as the genetic correlation. I derived the genetic correlations straight from my model ( $\rho_{\text{sire}}$ ) as the mean of the posterior distribution. To calculate the genetic variance, I first derived the posterior distributions of the standard deviations of the sire effect ( $\sigma_{\text{sire}}$ ) for every trait and dose combination. I then calculated the genetic variance posterior as the square of the sire effect standard deviation for each posterior sample. Finally, I calculated the mean genetic variance across all posterior samples for every trait dose combination. I calculated the genetic covariance by multiplying the sire effect's standard deviation for each trait and dose with the corresponding genetic correlation.

I used ggplot2 and ggridges (Wickham et al., 2019; Wilke, 2018) to visualize the whole posterior distributions for many parameters to check for strength, direction, and uncertainty of the estimates since the mean of the posterior distribution only provides a single point estimate. The code and data will be available for inspection via GitHub at [DeborahvanPutten/Code-data-master-thesis](https://github.com/DeborahvanPutten/Code-data-master-thesis).

## 4.0 Results

The final dataset included 9 sires and 16 dams, with an average of 1.8 dams mated per sire. For every treatment, 10 larvae were used, and a total of 60 larvae per dam. In total, there were 960 larvae in the final data set. To avoid bias from mortality unrelated to the biopesticide, for example, due to handling, dead larvae before day 3 after inoculation were excluded for analysis. In total, 916 Larvae made it past day 3 and were used for further analysis. Survival from day 3 till day 14 was high in all six different treatment groups with no apparent differences observed between the different groups including the control (figure 2). There was no indication that any of the doses led to an increased mortality after day 3 till day 14. Since the mortality rate was so low and did not conspicuously vary across doses, I conducted no formal genetic analyses on survival in the presence of the biopesticide. Further analyses on development rate and pupal mass were conducted on male larvae only, with a total of 416 larvae in the data set.

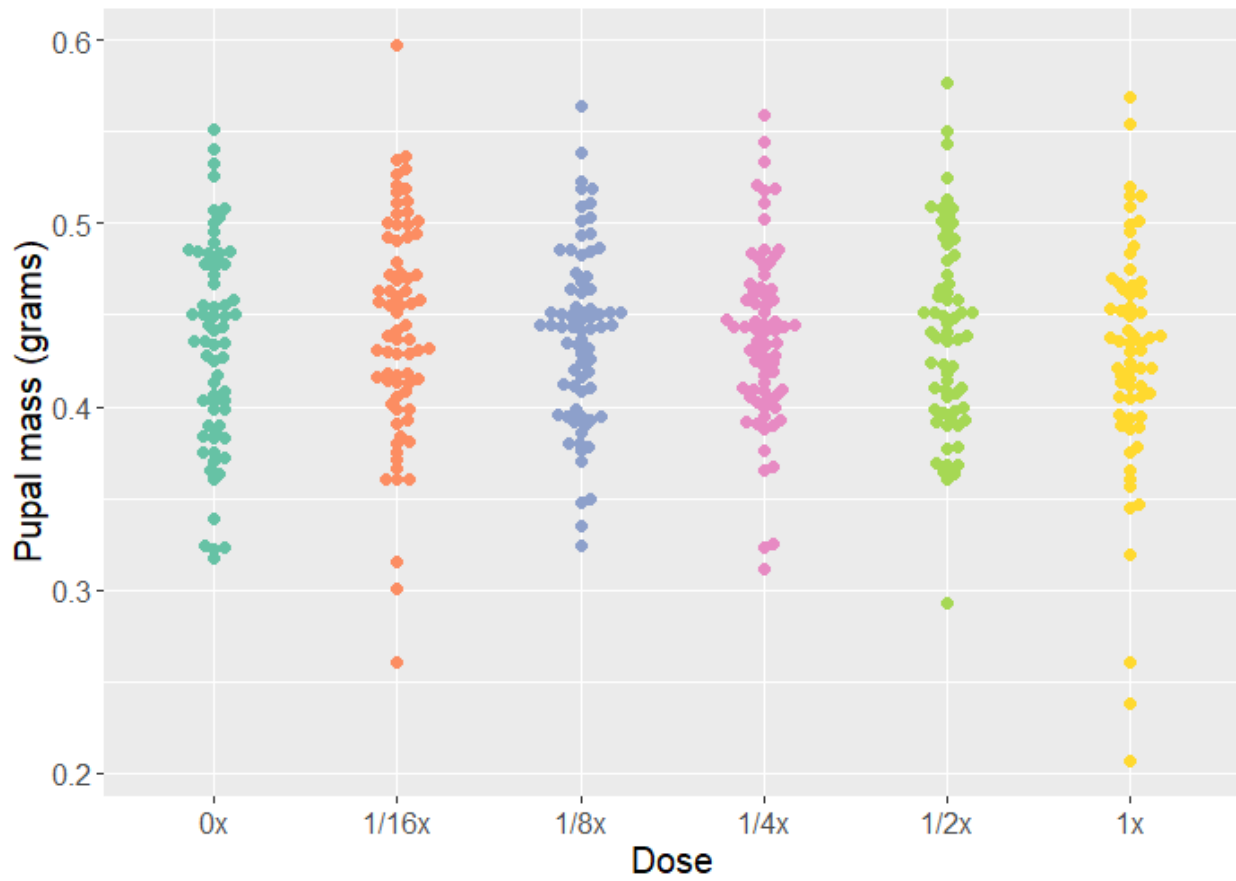


**Figure 2: Mortality proportion of larvae between day 3 and day 14 after inoculation with different doses of fungal biopesticide.** Each point represents the mortality proportion within that treatment group. Mortality proportion was calculated as the number of larvae that died in that dose in proportion to all larvae in that dose.

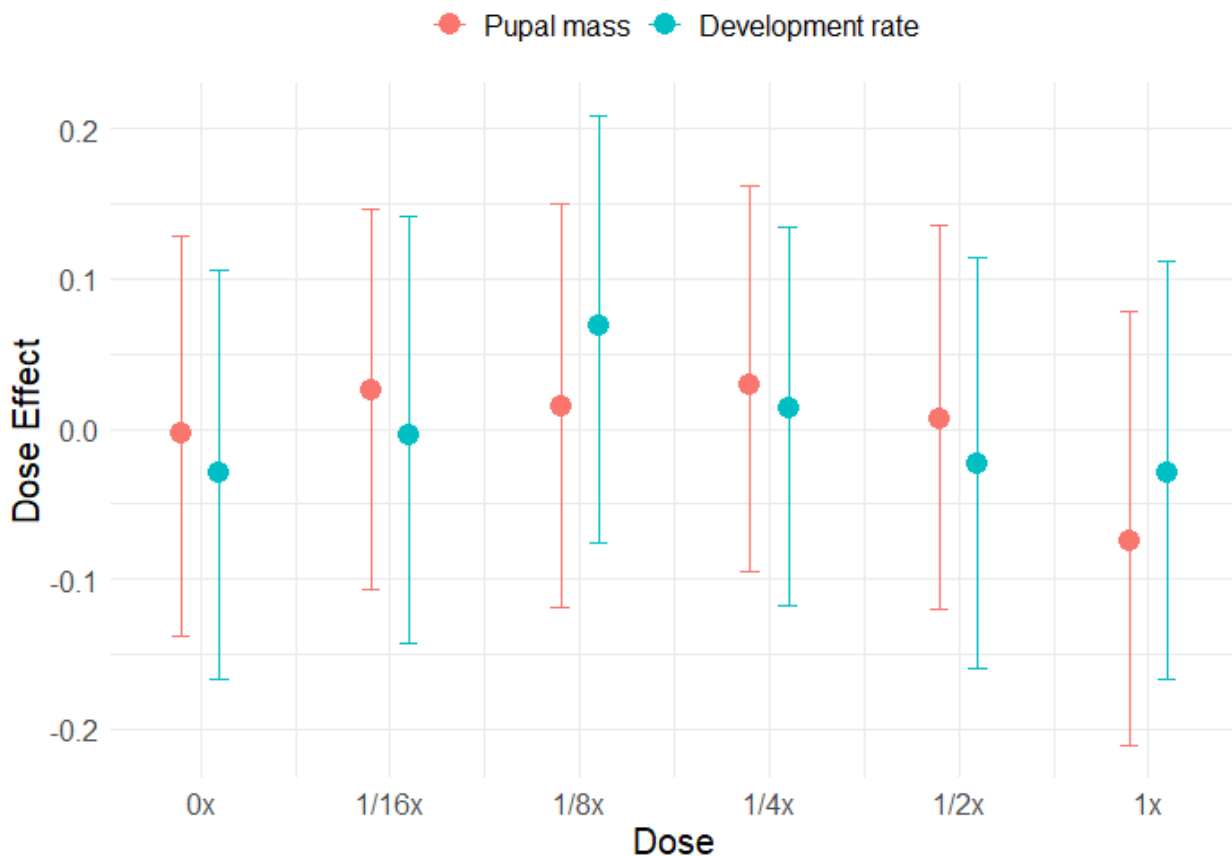
### 4.1 Pupal mass

Moths that survived beyond day 14 were kept for further analysis of pupal mass and development rate. To assess if pupal mass varied across doses, I first examined the distribution of pupal mass per dose (figure 3). Pupal mass distributions were similar across all doses.

To further investigate the dose-dependent effect of the pesticide, I estimated the dose effect of the pesticide on pupal mass using my Bayesian model (figure 4). The dose effect on pupal mass is shown in red. The median estimates were close to 0 with large 89% credible intervals spanning into both negative and positive values.



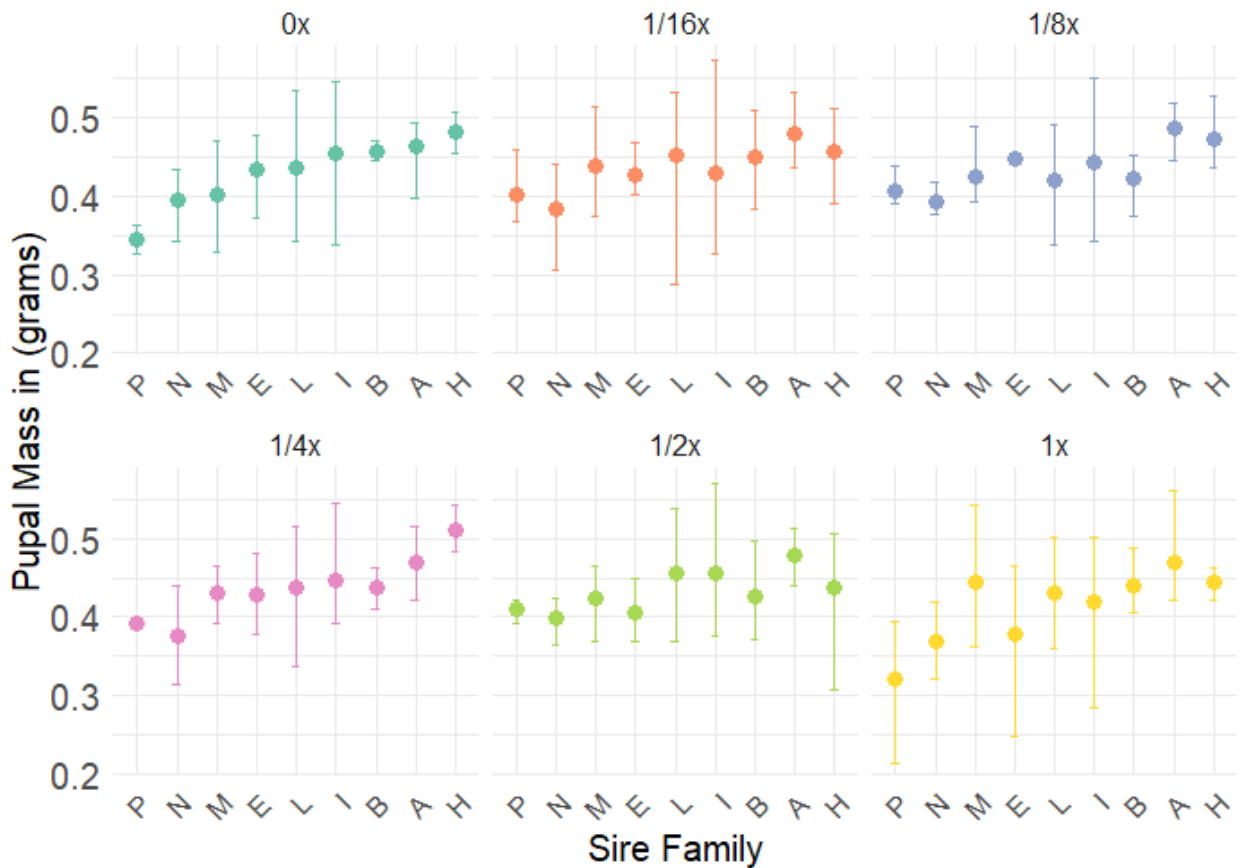
**Figure 3: Pupal mass distributions in male larvae across different biopesticide doses.** Each dot represents the pupal mass of an individual larva, visualized in a bee swarm to show the spread and density of the values.



**Figure 4: Estimated dose-dependent effect on pupal mass and development rate.** The estimated dose effect on pupal mass (red) and development rate (blue) were derived from a Bayesian model. The points are median values of the dose effect, and the bars are 89% credible intervals. The dose effect represents the deviation of the expected trait value (pupal mass and development rate) due to the biopesticide independent of genetic effects. This explains the dose-induced variation from the biopesticide. Trait values were log-transformed and standardized within each trait. As a result, the estimated dose effects are expressed in standard deviation units.

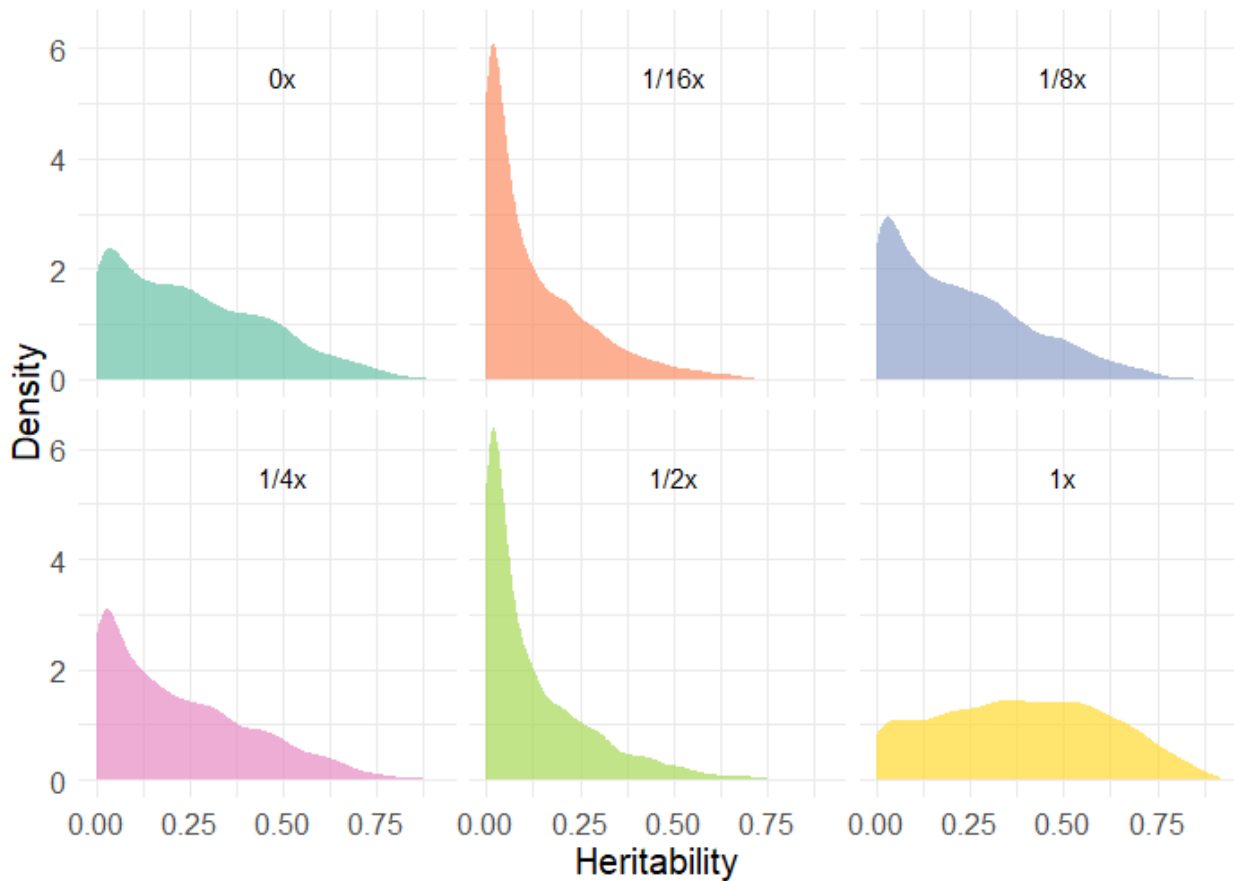
To explore potential genetic effects on pupal mass across doses, I first plotted sire family means across doses, ranking families by their pupal mass at the control dose (0x) (Figure 5). If there was a substantial genetic effect on pupal mass, I would expect some families to have higher pupal mass than others. Furthermore, if the effect of genes on mass also depends on dose, then I would expect the relative performance of families to shift across doses. For example, family 1 might have higher pupal mass than family 2 at a low dose, but lower pupal mass than family 2 at a higher dose.

When looking within dose, there is some variation in pupal mass between families. For example, family A and H consistently show a higher pupal mass than family P across every dose, indicating both a potential genetic difference. Moreover, since the relative performance between family A and P does not shift, neither is it obvious for other families, the genetic effect on pupal mass does not appear to depend on dose.



**Figure 5: Mean pupal mass (+/- 95% confidence interval) for each sire family across fungal doses.** Each plot represents a fungal dose in which the x-axis indicates sire identity, and the y axis shows pupal mass in grams. Each point within a plot represents the mean pupal mass for a sire family with 95% confidence intervals. To show potential gene-by-environment interactions, sires have been ranked on mass in response to the control dose 0x.

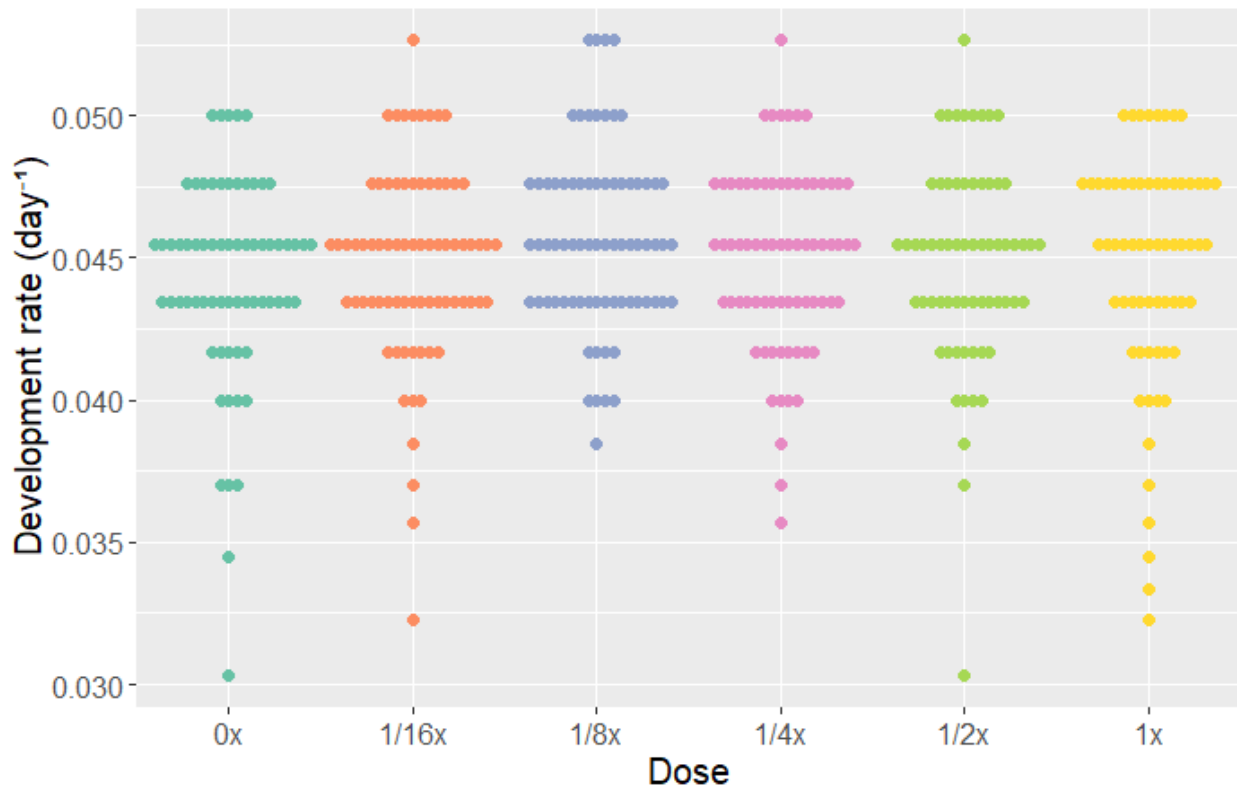
The previous analysis did not yet quantify genetic variation, because while close relatives share some genes, they are not clones. To better understand the genetic variation for pupal mass across doses, I estimated the narrow-sense heritability using the posterior distributions of the sire effects from my Bayesian model (figure 6). Some heritability distributions had a peak close to 0, but for all doses, the peak had wide tails, suggesting high uncertainty. In dose 1x, the posterior distribution was flat with a wide distribution. Because the posterior distribution showcases the range of credible heritabilities, its wide range supports both scenarios in which genetic variation is substantial as well as alternatives in which there is little genetic variation.



**Figure 6: Posterior distributions of heritability ( $h^2$ ) estimates of pupal mass in larvae across doses.** Each plot represents a different dose, with the x-axis showing narrow-sense heritability and the y-axis displaying the density.

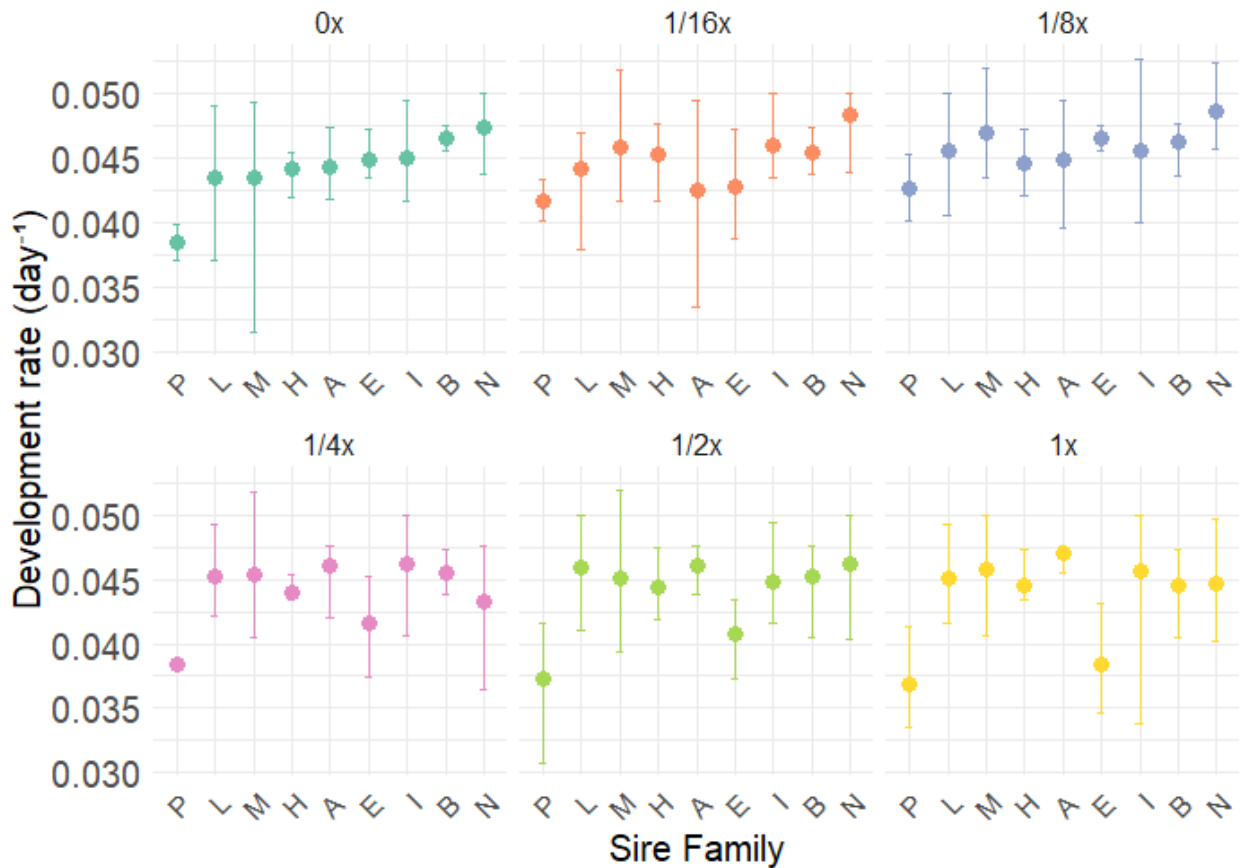
#### 4.2 Development rate

To assess whether the development rate changes across doses, I plotted the distribution of development rates in males per dose (figure 7). There were no conspicuous differences in the distributions of phenotypic development rates between the different doses. To further investigate the dose-dependent effect on development rate, I estimated the dose effect of the pesticide using my Bayesian model (figure 4). The dose effect on the development rate is depicted in blue. The median estimates were close to 0 with large overlapping credible intervals into both negative and positive values.



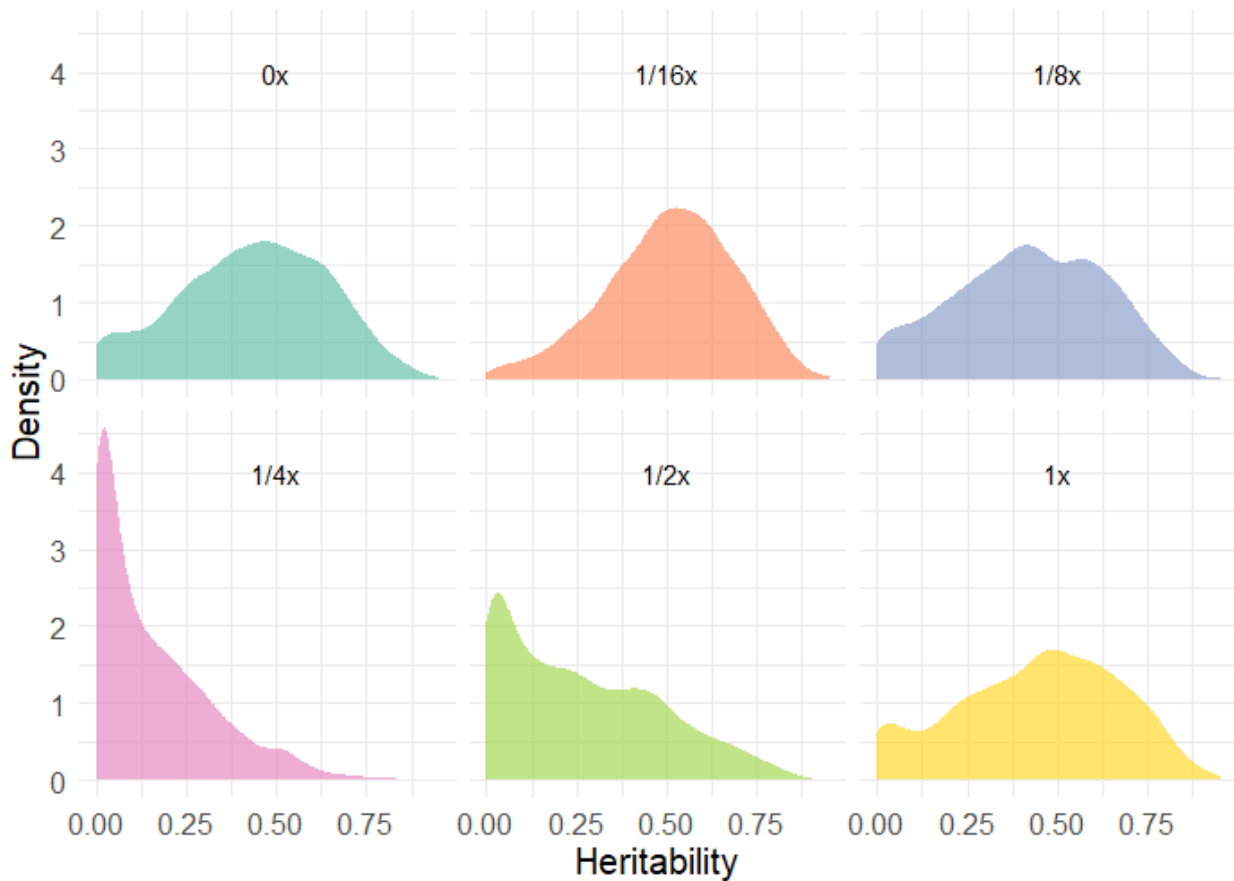
**Figure 7: Development rate distributions in larvae across different doses.** Each dot represents the development rate of an individual larva, visualized in a bee swarm to show the spread and density of the values. The development rate is calculated as the inverse of days from egg hatching to pupation.

To investigate any potential genetic effect on development rate across doses, I first plotted sire family means across doses, ranking families by their development rate performance at the control dose (0x) (Figure 8). As in pupal mass, if there was a genetic effect on the development rate, I would expect some families to have a higher development rate than other families. Additionally, if the effects of genes on development rate are influenced by dose, then I would expect the relative development rate between families to change across doses. The results show that within dose, there is some variation in the development rate between families. For example, family N has a higher development rate than family P, indicating potential genetic effects on the development rate. Between doses, the relative performance of development rate changes for some families, especially at higher doses, see for example family E. This suggests that genetic effects may vary across doses.



**Figure 8: Mean development rate (+/- 95% confidence interval) for each sire family across fungal doses.** Each plot represents a fungal dose in which the x-axis indicates sire identity, and the y axis shows development rate. Each point within a plot represents the mean development rate for a sire family with 95% confidence intervals. To show potential gene-by-environment interactions, sires have been ranked on development rate performance in response to the control dose 0x. The development rate is calculated as the inverse of days from egg hatching to pupation.

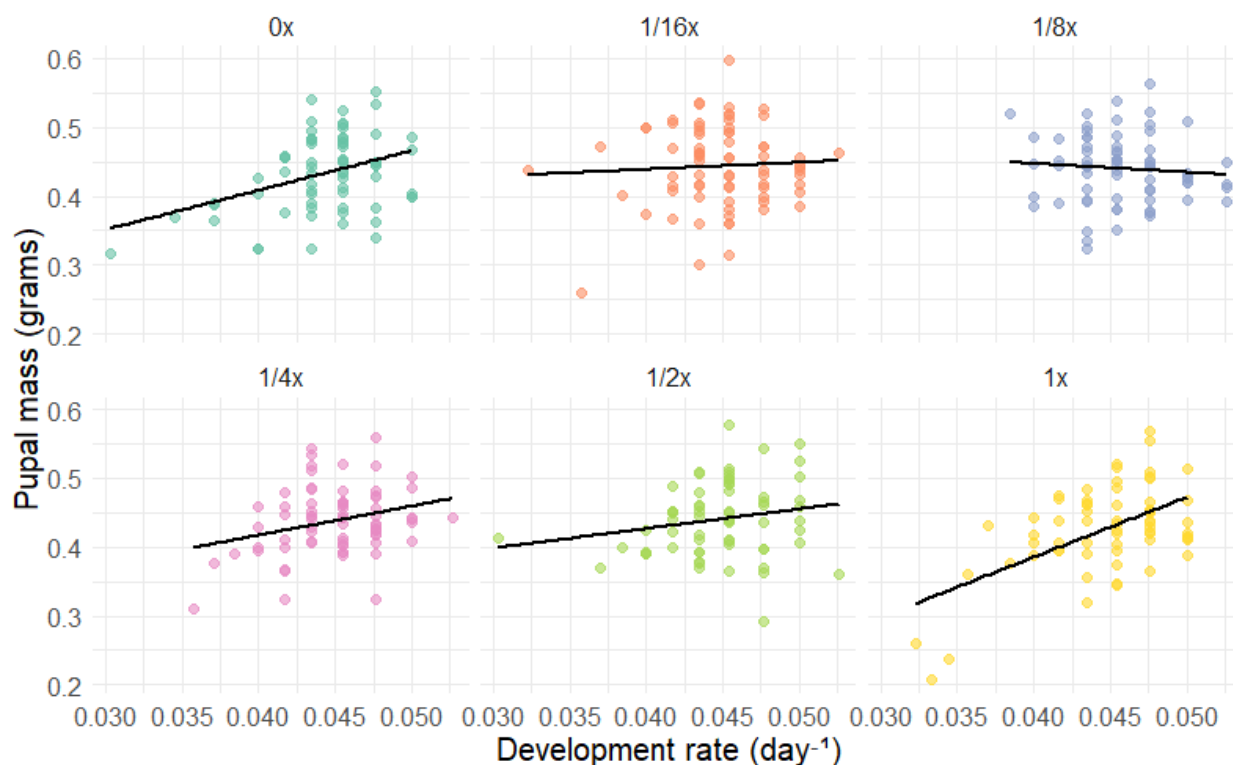
The previous analysis did not account fully for genetic effects as it still contains non-genetic variation. To better understand the genetic variation for development rate across doses, I estimated the narrow-sense heritability using the posterior distributions of the sire effect from my Bayesian model (figure 9). The posterior distributions of heritability vary across doses. Heritability estimates at doses 0x, 1/16x, 1/8x, and 1x show a wide posterior distribution with low peaks around heritability values of approximately 0.5. In contrast, heritability at doses 1/4x and 1/2x show posterior distributions with peaks closer to heritability values of 0, but with broad posterior tails indicating substantial uncertainty.



**Figure 9: Posterior distribution of heritability ( $h^2$ ) estimates of development rate across doses.** Each plot represents a different dose, with the x-axis showing narrow-sense heritability and the y-axis displaying the density.

### 4.3 Pupal mass by development rate

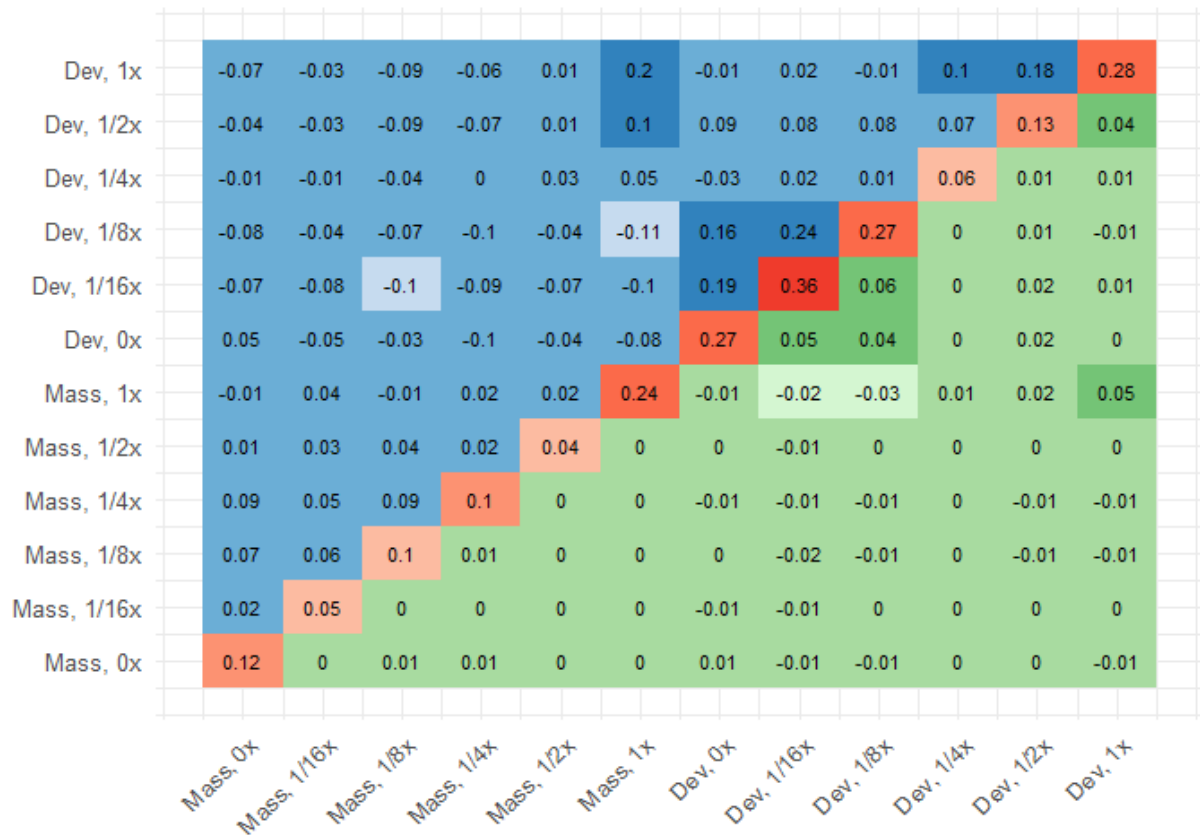
Therefore, it is important to consider the development rate in relation to the pupal mass to see how development time affects pupal mass. To assess this relationship, I plotted phenotypic associations between pupal mass against the development rate for each dose (figure 10). The relationships between pupal mass and development rate are in general positive. The highest genetic correlation is found at the highest dose, 1x.



**Figure 10: Relationship between pupal mass and development rate across doses.** Each plot represents a specific dose. Each dot in a plot represents an individual larva. The development rate is calculated as the inverse of days from egg hatching to pupation.

#### 4.5 G matrix

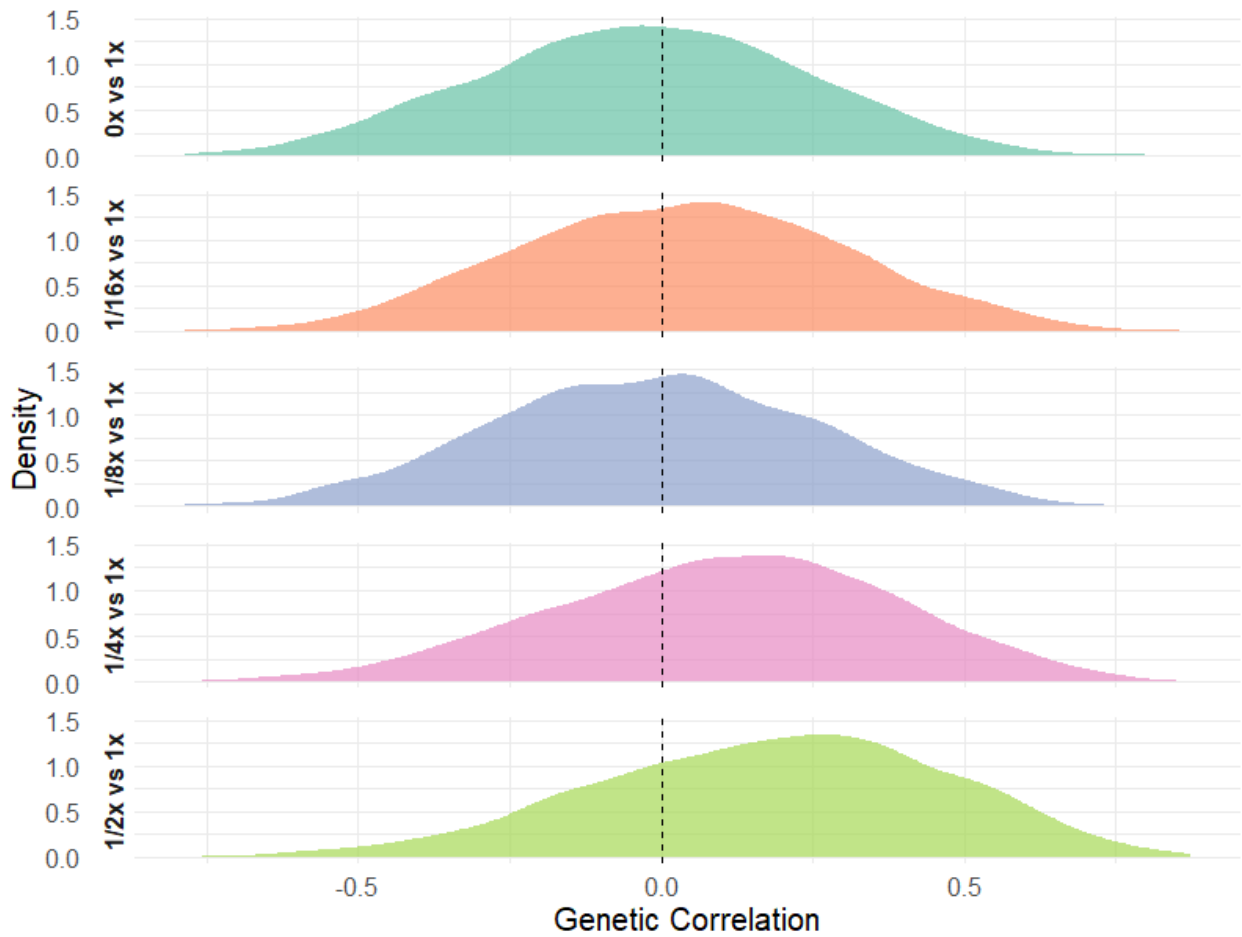
The previous analysis was a phenotypic analysis of the relationship between mass and development rate. Phenotypic relationships can be driven by environmental differences in resource acquisition that can mask evidence of genetic trade-offs. I created a G matrix to uncover potential genetic trade-offs and assess the genetic variation between traits within and between doses. The G matrix contains a mean single-point summary of the entire posterior. Figure 11 shows the G matrix with genetic variances (red), correlations (blue), and covariances (green). The genetic variances for development rate were higher than for pupal mass across most doses.



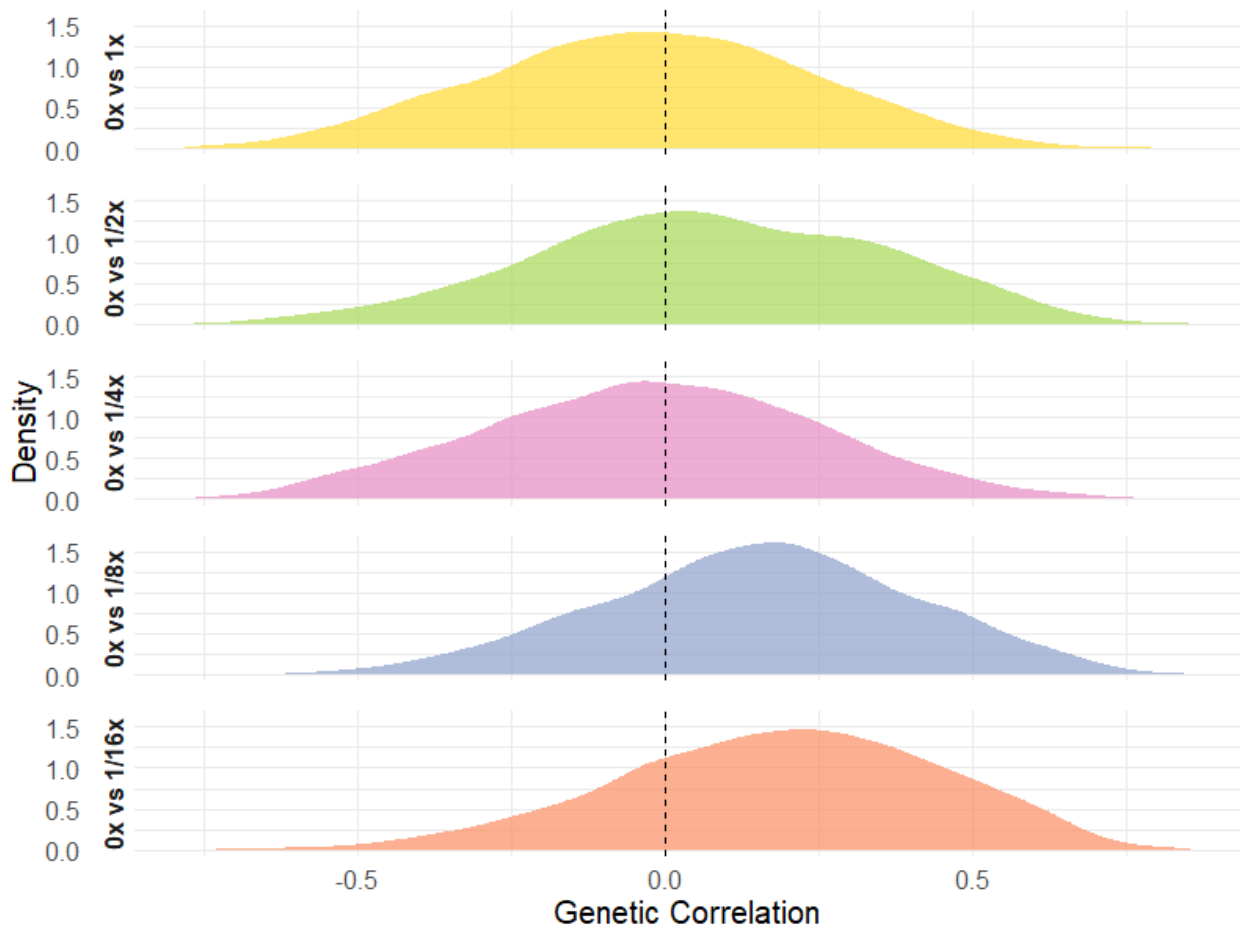
**Figure 11: G matrix for pupal mass and development rate in larvae across dose.** Each trait-dose combination is treated as a separate trait, labeled on both axes as “Mass” (pupal mass) and “Dev” (development rate) for every dose. The G matrix contains the genetic variances (red), genetic co-variances (green), and genetic correlations (blue) summarized using mean values from the posterior. However, note that the entire posterior is the best representation of evidence, and that the model supported substantial uncertainty in these measures (see figures 12, 13 and 14 for distributions of genetic correlations).

The mean point summaries of genetic correlations across development rates and pupal mass and different doses were generally low. The genetic correlations across doses within pupal mass were also low and close to 0. However, in the development rate in males, there were slight increases in genetic correlations among similar doses (i.e. dose 0x and 1/16x, dose 1/8x and 1/16x, dose 1x and dose 1/2x). These genetic correlations decreased with increased dose difference.

Since the values used in the G matrices are mean point summaries of the entire posterior distribution, it does not capture the full range of values within the posterior distribution. Therefore, it is also important to look at the full posterior distributions of the genetic correlations. Figure 12 shows the posterior distributions of genetic correlations between development rate under dose 1 with development rate in each of the other doses. This plot showcases how the genetic correlations change with increasing dose differences. The top plot shows the genetic correlation between dose 1x and 0x, which is centered around 0. Moving down the figure, the dose comparisons with dose 1x moves along the dose-gradient towards increasingly similar doses. Towards similar doses, the center of the posterior distributions moves in a positive direction. The same is true when comparing dose 0x with all other doses (figure 13). The top plot reveals the genetic correlation across the most similar doses 0x and 1.16x, and following down the plots the dose difference increases and the center of the posterior moves towards 0. However, for both genetic correlation plots 12 and 13, all posterior distributions are wide, indicating high uncertainty.

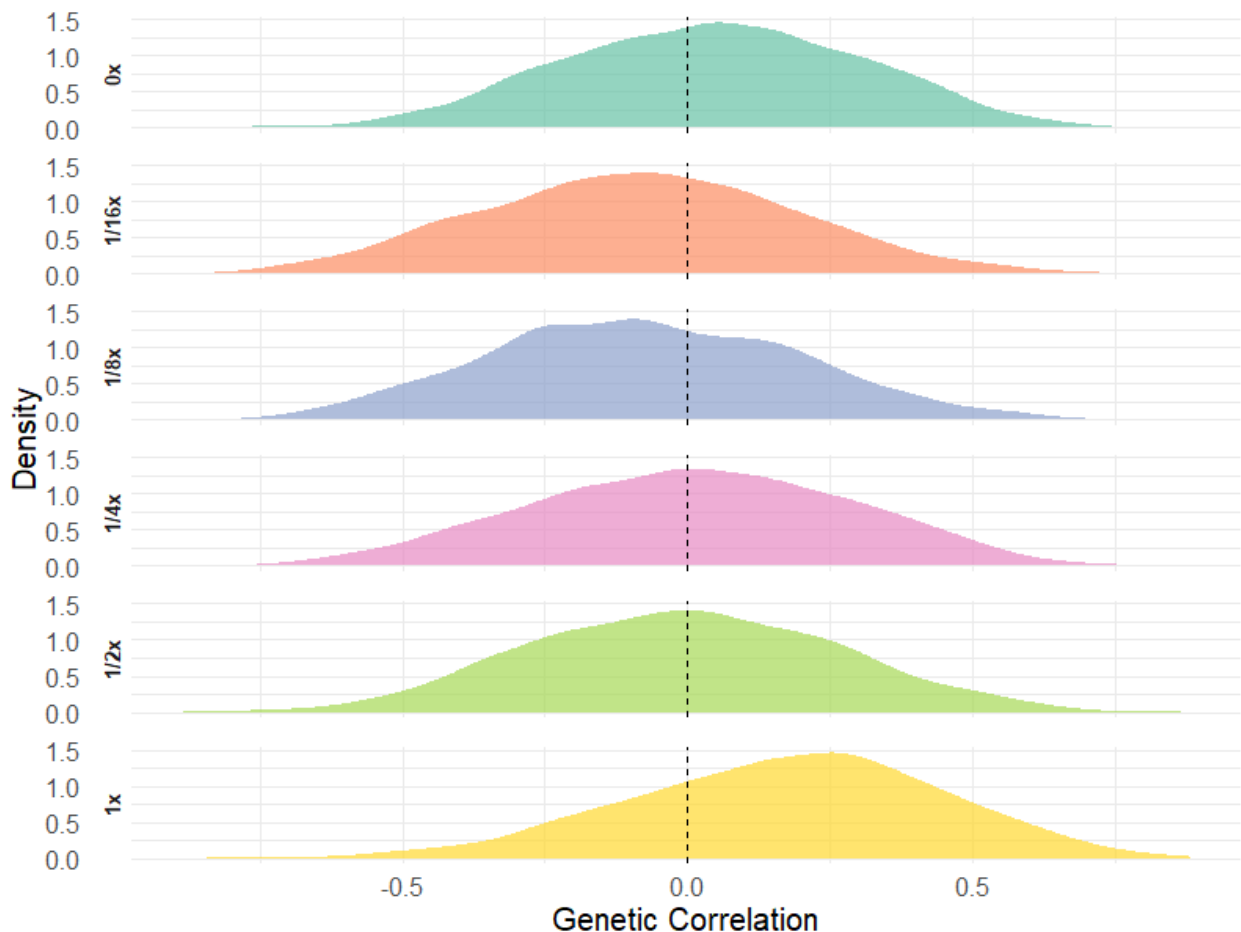


**Figure 12: Posterior distributions of genetic correlations between dose 1x and the other doses in development rate.** The dose difference is the highest in the top plot (dose 1x vs. 0x), and the lowest in the bottom plot (dose 1x vs. 1/2x). The dashed line marks the 0.0 genetic correlation point.



**Figure 13: Posterior distributions of genetic correlations between dose 0x and the other doses in development rate.** The dose difference is the highest in the top plot (dose 0x vs. 1x), and the lowest in the bottom plot (dose 0x vs. 1/16x). The dashed line marks the 0.0 genetic correlation point.

When comparing pupal mass and development rate within the same dose in the G matrix, most mean genetic correlations values are close to 0 or slightly negative. However, for dose 1x, there is a noticeable higher positive correlation between pupal mass and development rate. Figure 14 shows the posterior distributions of the genetic correlation between pupal mass and development rate for every dose. Dose 1x (yellow) shows a skew towards more positive values, with a peak around 0.2, in contrast to the distributions of the other doses with a peak around 0 or even negative values. Even though the majority of the posterior distribution at dose 1x lies in the positive range, the distribution is broad and overlaps into negative values, indicating high uncertainty.



**Figure 14: Posterior distributions of genetic correlation between pupal mass and development rate across all doses.** The top plot represents dose 0x and increases in dose towards the bottom plot of dose 1x. The dashed line marks the 0.0 genetic correlation point.

## 6.0 Discussion

Host-pathogen interactions, as seen in pest and biopesticide resistance evolution, are often influenced by genetic trade-offs between life-history traits (Lazzaro & Little, 2008). The way that organisms allocate resources determines the trade-off, but it can nevertheless be masked by variation in resource acquisition (Van Noordwijk & De Jong, 1986). Additionally, a changing environment can influence the complex interactions between resource acquisition and allocation, which can in turn influence how selection acts on genetic variation. While different biopesticide doses are known to have varying phenotypic effects on life-history traits (Wakil et al., 2022), less is known about the genetic responses predicted for these effects. The aim of this thesis was to quantify genetic parameters across life-history traits and environments. Specifically, I aimed to discover if dose-dependent genetic variation can constrain evolution. I tried to do this by looking at: (1) within-trait genetic correlations across doses to determine GxE interactions, and (2) between-trait genetic correlation within doses to see if resource acquisition or allocation influences trait values, and the extent to which this changes across doses.

The low mortality and the lack of a dose-dependent effect on pupal mass and development rate suggest that on a phenotypic level, the biopesticide did not strongly affect larvae, though this does not rule out the possibility of genetic variation in these traits as they can be hidden by environmental effects or by genetic variation in resource acquisition.

To understand the potential influence of resource allocation and acquisition, I looked at the genetic correlation between pupal mass and development rate. My results revealed opposing results in the

relationship between phenotypic and genetic relationship between pupal mass and development rate. On a phenotypic level, the relationships were mostly positive, but the Bayesian model revealed many slightly negative genetic correlations between the two traits. Some of these differences may be due to environmental influences, since the phenotypic relationship contains both the genetic and environmental effects, while the genetic analysis only contains the genetic effects. However, these differences between phenotypic and genetic covariances are often observed between life-history traits and may underly genetic variation in resource acquisition and allocation (Reznick et al., 2000; Van Noordwijk & De Jong, 1986). If only genetic variation in resource allocation influenced the relationship between these two traits, I would expect strong negative correlations, since investment in one trait (e.g. development rate) cannot be spent on the other trait (e.g. pupal mass). In contrast, if only genetic variation in resource acquisition influenced the relationship between these two traits, I would expect strong positive genetic correlations, as genotypes with greater resource acquisition can spend on both traits. My results are consistent with two possibilities. First, it could be that there are low levels of genetic variation in acquisition and allocation. However, this would be very surprising: the population I study is from an extremely large industrial population used to culture baculovirus, and previous experiments have demonstrated that it contains a lot of genetic variation in life history traits (Mangan et al., 2025). More probably, my results suggest that both genetic variation in resource acquisition and allocation are influencing both traits, and that positive effects of genetic variation for acquisition is balanced by negative patterns imposed by genetic trade-offs.

Interestingly, the highest genetic correlation between development rate and pupal mass was found within the highest dose, 1x, while lower doses showed genetic correlations closer to 0. Although the posterior distributions for all those genetic correlations were wide, indicating high uncertainty, dose 1x was considerably skewed towards higher values, suggesting evidence for a stronger positive genetic association between development rate and pupal mass. A positive correlation at the highest dose indicates that genetic factors influenced both fast development and high pupal mass. High-stress environments, like in the highest dose, can potentially limit resources for larvae to use. Normally in the limited resource model of trade-offs, a negative correlation would be expected, which is not observed here. The positive genetic correlation in the highest dose might mean that when resources become more limited and harder to acquire, genetic variation for resource acquisition has relatively more influence on both traits than genetic variation for resource allocation.

The low genetic correlations in lower doses can potentially be caused by reduced environmental stress. Low-stress environments can mask genetic variation due to favorable conditions providing low selective pressures on resource acquisition and allocation (Hoffmann & Merilä, 1999; Sgrò & Hoffmann, 2004). This can mean that all larvae, regardless of genetic background, can acquire enough resources to grow both big and fast, leading to a positive phenotypic correlation, but no genetic correlations. In this scenario, genetic correlations between life-history traits are hard to detect as differences in trait performance do not depend heavily on genetic factors. Indeed, several studies have shown that genetic variation can be masked in low-stress environments and become more apparent in high-stress environments (Hillesheim & Stearns, 1991; Howie et al., 2019; Imasheva et al., 1998). Notwithstanding the uncertainty from the model, these results suggest that GxE interactions can potentially influence the role of genetic variation for resource acquisition on trait performance.

When looking at the genetic parameters within traits, the Bayesian model revealed higher genetic variation in development rate than in pupal mass. Additionally, there was evidence for GxE interactions in development rate when comparing low and high doses with the other doses. The smaller the environmental distance in dose, the higher the genetic correlation, and vice versa. This pattern suggests that certain genotypes that perform well in one certain dose, do not necessarily perform well in a different dose, hinting at genetic trade-offs driven by GxE interactions. My results are therefore consistent with those of Schou et al. (2019), who provided evidence from livestock that genetic correlations degrade as environmental distance increases.

The extent to which genetic trade-offs are driven by GxE interactions will affect how varying environmental conditions can maintain genetic diversity in a population by preventing directional selection

towards one favorable genotype. From an applied perspective, the observed dose-dependent genetic effects could have important implications for farmers managing pests and resistance evolution. Agricultural landscapes tend to be highly homogeneous, and although it has been argued before that increasing the environmental variability in agricultural landscapes can be beneficial for pest resistance management (Mangan et al., 2023), implementing it is not always easy due to economic costs, or logistics. Farmers are running a business, and what is best for pest resistance management from a biological standpoint, is not always best for them from a business standpoint. Farmers' decisions are affected by profitability and market demands, so finding sustainable management approaches that insulate farmers from economic losses is crucial. My thesis showed that variation in biopesticide doses alone may be able to disrupt selection on pest insects. This finding is useful because farmers can easily achieve dose variation using current methods: the spatial variation in product application, coupled to variation in pest target behavior, location, and developmental stage will all contribute to heterogeneous exposure to pathogen risk. However, dose variation alone may not be sufficient for pest resistance management. It is most likely the accumulation of several sources of environmental variability that can make the biggest impact on slowing down resistance evolution (Bourguet et al., 2012). A complex heterogeneous environment will create multiple fluctuating selection pressures by continuously altering the fitness landscape in the pest population. For this reason, future research into other easily applicable methods to induce environmental variation is needed to effectively constrain resistance evolution in pests.

An important limitation of this study is the limited pesticide treatment effect on the measured traits, as mortality was low. A possible explanation is that the experimental conditions were not stressful enough. The larvae were fed an artificial, sterilized diet optimized for growth by providing all necessary nutrients in optimal quantities. In contrast, larvae in natural agricultural environments feed on natural resources, such as leaves or seeds, which can often be less optimal in nutrient availability. Previous research has shown that *Helicoverpa armigera* larvae have higher survival and development rates on artificial diets than on natural plants like cotton, maize and soybean (Da Silva et al., 2020). Furthermore, in the wild, larvae face other environmental stressors such as weather variability, competition, pathogens, and predators. These factors put more stress on the larvae and can induce greater susceptibility to the biopesticide compared to those larvae kept in a controlled laboratory environment. For example, the importance of stress was apparent in a study on the ladybird beetle *Harmonia axyridis* in which genetic variation in body mass was only unmasked under nutritional stressful conditions, but was undetected in a high food environment (Dmitriew et al., 2010). Furthermore, a study on immune defense in bumblebees showed that individuals with ad libitum feeding had no change in survival, while starving individuals had a drastic drop in survival in response to immune defense activation (Moret & Schmid-Hempel, 2000). Similarly, it is possible that the artificial diet may have led to masked genetic variation in pupal mass and possibly development rate, which could explain the high uncertainty and lack of clear genetic effects.

Another explanation of the broad posterior distributions extracted from the model indicating high uncertainty is the low number of sire families. The final data set was large in terms of individually housed caterpillars, but due to constraints on lab space and processing time, it included only 9 sires, with 16 full sibling families in total. Some sires mated only once due to mating difficulties in laboratory settings (similar to previous attempts for quantitative genetics in this species (Mangan et al., 2025)). In half-sibling designs, the power to successfully detect additive genetic variation comes primarily from the number of sires mated with multiple dams, as variation among sires is needed to partition the additive genetic variation according to sire identity (Kolbehdari et al., 2005). Furthermore, each treatment group within a family and dose included 10 larvae. For analyses of mass and development time, I further split the data into same-sex groups to account for known sex-dependent differences in these traits. This and some early handling-related mortality meant that some family sex-dose groups only included 1 larva, which likely further increased the uncertainty of the estimates. Therefore, for future studies it is advisable to increase the number of sires (split across multiple blocks to protect the welfare of the researchers) as well as including more offspring per treatment group.

Lastly, it should be noted that there might be genetic differences between populations, which suggests caution in interpreting findings from a single population (Roff, 1996). However, Weigensberg and

Roff (1996) showed that heritabilities across laboratory or wild populations are often strikingly consistent, indicating that laboratory estimates of genetic variation and covariation can reasonably represent wild populations.

This thesis aimed to quantify genetic parameters between and within pupal mass and development rate across different doses. While the biopesticide showed limited effect at a phenotypic level, the Bayesian model suggested some underlying genetic variation and dose-dependent trade-offs, particularly for development rate. Moreover, the shift in genetic correlations between traits across different doses suggests that selection on resource acquisition depends on the level of pathogen exposure. These results show that genotype-by-environment interactions can help maintain genetic variation by preventing any single genotype from dominating. This mechanism may be particularly relevant in agricultural pest management, where sustaining genetic diversity could slow the evolution of pesticide resistance.

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## 8.0 References

- Agrawal, A. A. (2019). A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology*, *101*(2). <https://doi.org/10.1002/ecy.2924>
- Armbruster, P., & Hutchinson, R. A. (2002). Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *Journal of Medical Entomology*, *39*(4), 699–704. <https://doi.org/10.1603/0022-2585-39.4.699>
- Armes, N. J., Bond, G. S., & Cooter, R. J. (1992). *The laboratory culture and development of Helicoverpa armigera* (Bulletin No. 57). Natural Resources Institute.
- Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Frontiers in Microbiology*, *14*. <https://doi.org/10.3389/fmicb.2023.1040901>
- Blanford, S., Thomas, M. B., Pugh, C., & Pell, J. K. (2002). Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. *Ecology Letters*, *6*(1), 2–5. <https://doi.org/10.1046/j.1461-0248.2003.00387.x>
- Bourguet, D. D., Delmotte, F. F., Franck, P. P., Guillemaud, T., Reboud, X., Vacher, C. C., & Walker, A. S. a. S. (2012). Heterogeneity of selection and the evolution of resistance. *Trends in Ecology & Evolution*, *28*(2), 110–118. <https://doi.org/10.1016/j.tree.2012.09.001>
- B rger, R., & Gimelfarb, A. (2002). Fluctuating environments and the role of mutation in maintaining quantitative genetic variation. *Genetics Research*, *80*(1), 31–46. <https://doi.org/10.1017/s0016672302005682>
- Carthy, U. M., Uysal, I., Badia-Melis, R., Mercier, S., O'Donnell, C., & Ktenioudaki, A. (2018). Global food security – Issues, challenges and technological solutions. *Trends in Food Science & Technology*, *77*, 11–20. <https://doi.org/10.1016/j.tifs.2018.05.002>

- Chen, C., Xia, Q., Xiao, H., Xiao, L., & Xue, F. (2014). A comparison of the life-history traits between diapause and direct development individuals in the cotton bollworm, *Helicoverpa armigera*. *Journal of Insect Science*, *14*(1). <https://doi.org/10.1093/jis/14.1.19>
- Cohen, A. A., Coste, C. F. D., Li, X., Bourg, S., & Pavard, S. (2019). Are trade-offs really the key drivers of ageing and life span? *Functional Ecology*, *34*(1), 153–166. <https://doi.org/10.1111/1365-2435.13444>
- Da Silva, I. F., Baldin, E. L. L., Specht, A., Roque-Specht, V. F., Morando, R., Malaquias, J. V., & Paula-Moraes, S. V. (2020). Role of nutritional composition in the development and survival of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on artificial diet and natural hosts. *Bulletin of Entomological Research*, *111*(3), 257–269. <https://doi.org/10.1017/s0007485320000449>
- Dmitriew, C., Blows, M. W., & Rowe, L. (2010). Ontogenetic change in genetic variance in size depends on growth environment. *The American Naturalist*, *175*(6), 640–649. <https://doi.org/10.1086/652470>
- Falconer, D. S. (1989). *Introduction to Quantitative Genetics* (3rd ed.). Longman Scientific and Technical.
- Ffrench-Constant, R. H. (2013). The molecular genetics of insecticide resistance. *Genetics*, *194*(4), 807–815. <https://doi.org/10.1534/genetics.112.141895>
- Garland, T., Downs, C. J., & Ives, A. R. (2021). Trade-offs (and constraints) in organismal biology. *Physiological and Biochemical Zoology*, *95*(1), 82–112. <https://doi.org/10.1086/717897>
- Haave-Audet, E., Besson, A. A., Nakagawa, S., & Mathot, K. J. (2021). Differences in resource acquisition, not allocation, mediate the relationship between behaviour and fitness: a systematic review and meta-analysis. *Biological Reviews/Biological Reviews of the Cambridge Philosophical Society*, *97*(2), 708–731. <https://doi.org/10.1111/brv.12819>
- Haile, F., Nowatzki, T., & Storer, N. (2020). Overview of pest status, potential risk, and management considerations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) for U.S. soybean production. *Journal of Integrated Pest Management*, *12*(1). <https://doi.org/10.1093/jipm/pmaa030>
- Hillesheim, E., & Stearns, S. C. (1991). The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypic plasticity in two larval food environments. *Evolution*, *45*(8), 1909–1923. <https://doi.org/10.1111/j.1558-5646.1991.tb02696.x>
- Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, *14*(3), 96–101. [https://doi.org/10.1016/s0169-5347\(99\)01595-5](https://doi.org/10.1016/s0169-5347(99)01595-5)
- Holt, R. D., Hochberg, M. E., & Barfield, M. (1999). Population dynamics and the evolutionary stability of biological control. In *Cambridge University Press eBooks* (pp. 219–230). <https://doi.org/10.1017/cbo9780511542077.018>
- Honěk, A., & Honek, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, *66*(3), 483. <https://doi.org/10.2307/3544943>
- Howie, J. M., Dawson, H. a. C., Pomiankowski, A., & Fowler, K. (2019). Limits to environmental masking of genetic quality in sexual signals. *Journal of Evolutionary Biology*, *32*(8), 868–877. <https://doi.org/10.1111/jeb.13491>
- Imasheva, A. G., Loeschke, V., Zhivotovsky, L. A., & Lazebny, O. E. (1998). Stress temperatures and quantitative variation in *Drosophila melanogaster*. *Heredity*, *81*(3), 246–253. <https://doi.org/10.1046/j.1365-2540.1998.00384.x>
- Jessup, C. M., & Bohannan, B. J. M. (2008). The shape of an ecological trade-off varies with environment. *Ecology Letters*, *11*(9), 947–959. <https://doi.org/10.1111/j.1461-0248.2008.01205.x>

- Kasamatsu, E., & Abe, J. (2015). Influence of body size on fecundity and sperm management in the parasitoid wasp *Anisopteromalus calandrae*. *Physiological Entomology*, 40(3), 223–231. <https://doi.org/10.1111/phen.12106>
- King, E. G., Roff, D. A., & Fairbairn, D. J. (2010). Trade-off acquisition and allocation in *Gryllus firmus*: a test of the Y model. *Journal of Evolutionary Biology*, 24(2), 256–264. <https://doi.org/10.1111/j.1420-9101.2010.02160.x>
- Kolbehdari, D., Jansen, G. B., Schaeffer, L. R., & Allen, B. O. (2005). Power of QTL detection by either fixed or random models in half-sib designs. *Genetics Selection Evolution*, 37(7). <https://doi.org/10.1186/1297-9686-37-7-601>
- Kraaijeveld, A. R., Ferrari, J., & Godfray, H. C. J. (2002). Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology*, 125(7), S71–S82. <https://doi.org/10.1017/s0031182002001750>
- Lazzaro, B. P., & Little, T. J. (2008). Immunity in a variable world. *Philosophical Transactions of the Royal Society B Biological Sciences*, 364(1513), 15–26. <https://doi.org/10.1098/rstb.2008.0141>
- Liu, X., Cao, A., Yan, D., Ouyang, C., Wang, Q., & Li, Y. (2019). Overview of mechanisms and uses of biopesticides. *International Journal of Pest Management*, 67(1), 65–72. <https://doi.org/10.1080/09670874.2019.1664789>
- Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, 88(1), 87–98. <https://doi.org/10.1034/j.1600-0706.2000.880110.x>
- Mangan, R., Bussière, L. F., Polanczyk, R. A., & Tinsley, M. C. (2023). Increasing ecological heterogeneity can constrain biopesticide resistance evolution. *Trends in Ecology & Evolution*, 38(7), 605–614. <https://doi.org/10.1016/j.tree.2023.01.012>
- Mangan, R. M., Tinsley, M. C., Ferrari, E., Polanczyk, R. A., & Bussière, L. F. (2025). Crop diversity induces trade-offs in microbial biopesticide susceptibility that could delay pest resistance evolution. *PLoS Pathogens*, 21(5), e1013150. <https://doi.org/10.1371/journal.ppat.1013150>
- McElreath, R. (2020a). *Statistical rethinking: A Bayesian course with examples in R and Stan*. Chapman & Hall/CRC.
- McElreath, R. (2020b). *rethinking: Statistical Rethinking package (Version 2.13)* [R package]. GitHub. <https://github.com/rmcelreath/rethinking>
- Moiron, M., Laskowski, K. L., & Niemelä, P. T. (2019). Individual differences in behaviour explain variation in survival: a meta-analysis. *Ecology Letters*, 23(2), 399–408. <https://doi.org/10.1111/ele.13438>
- Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: The price of immune system activation for Bumblebee workers. *Science*, 290(5494), 1166–1168. <https://doi.org/10.1126/science.290.5494.1166>
- Nylin, S., & Gotthard, K. (1998). Plasticity in Life-History traits. *Annual Review of Entomology*, 43(1), 63–83. <https://doi.org/10.1146/annurev.ento.43.1.63>
- Orke, E., & Dehne, H. (2003). Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection*, 23(4), 275–285. <https://doi.org/10.1016/j.cropro.2003.10.001>
- R Core Team. (2024). *R: A language and environment for statistical computing* (Version 4.4.1) [Computer software]. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Reznick, D., Nunney, L., & Tessier, A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution*, 15(10), 421–425. [https://doi.org/10.1016/s0169-5347\(00\)01941-8](https://doi.org/10.1016/s0169-5347(00)01941-8)

- Roff, D. A., & Mousseau, T. A. (1987). Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity*, 58(1), 103–118. <https://doi.org/10.1038/hdy.1987.15>
- Royauté, R., Berdal, M. A., Garrison, C. R., & Dochtermann, N. A. (2018). Ppaceless life? A meta-analysis of the pace-of-life syndrome hypothesis. *Behavioral Ecology and Sociobiology*, 72(3). <https://doi.org/10.1007/s00265-018-2472-z>
- Schou, M. F., Hoffmann, A. A., & Kristensen, T. N. (2019). Genetic correlations and their dependence on environmental similarity—Insights from livestock data. *Evolution*, 73(8), 1672–1678. <https://doi.org/10.1111/evo.13762>
- Sgrò, C. M., & Hoffmann, A. A. (2004). Genetic correlations, trade-offs and environmental variation. *Heredity*, 93(3), 241–248. <https://doi.org/10.1038/sj.hdy.6800532>
- Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, 11(8), 317–321. [https://doi.org/10.1016/0169-5347\(96\)10039-2](https://doi.org/10.1016/0169-5347(96)10039-2)
- Sibly, R., & Calow, P. (1986). Why breeding earlier is always worthwhile. *Journal of Theoretical Biology*, 123(3), 311–319. [https://doi.org/10.1016/s0022-5193\(86\)80246-6](https://doi.org/10.1016/s0022-5193(86)80246-6)
- Sironi, M., Cagliani, R., Forni, D., & Clerici, M. (2015). Evolutionary insights into host–pathogen interactions from mammalian sequence data. *Nature Reviews Genetics*, 16(4), 224–236. <https://doi.org/10.1038/nrg3905>
- Stan Development Team. (2024). *RStan: The R interface to Stan* (Version 2.32.7) [R package]. <https://mc-stan.org/>
- Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology*, 3(3), 259. <https://doi.org/10.2307/2389364>
- Steinwascher, K. (1982). Relationship between pupal mass and adult survivorship and fecundity for *Aedes aegypti*. *Environmental Entomology*, 11(1), 150–153. <https://doi.org/10.1093/ee/11.1.150>
- Tinsley, M. C., Blanford, S., & Jiggins, F. M. (2006). Genetic variation in *Drosophila melanogaster* pathogen susceptibility. *Parasitology*, 132(6), 767–773. <https://doi.org/10.1017/s0031182006009929>
- United Nations, Department of Economic and Social Affairs, Population Division. (2024). World population prospects 2024: Ten key messages.
- Van Noordwijk, A. J., & De Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, 128(1), 137–142. <https://doi.org/10.1086/284547>
- Via, S. (1987). Genetic constraints on the evolution of phenotypic plasticity. In *Springer eBooks* (pp. 47–71). [https://doi.org/10.1007/978-3-642-72770-2\\_4](https://doi.org/10.1007/978-3-642-72770-2_4)
- Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39(3), 505–522. <https://doi.org/10.1111/j.1558-5646.1985.tb00391.x>
- Wakil, W., Ghazanfar, M. U., Usman, M., Hunter, D., & Shi, W. (2022). Fungal-based biopesticide formulations to control nymphs and adults of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae): A Laboratory and Field Cage Study. *Agronomy*, 12(5), 1160. <https://doi.org/10.3390/agronomy12051160>
- Weigensberg, I., & Roff, D. A. (1996). Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution*, 50(6), 2149–2157. <https://doi.org/10.1111/j.1558-5646.1996.tb03605.x>

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Golemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., ... Yutani, H. (2019). *Welcome to the tidyverse*. *Journal of Open Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>

Wilke, C. O. (2018). *ggridges: Ridgeline plots in ggplot2* (R package version 0.5.0) [Computer software]. <https://CRAN.R-project.org/package=ggridges>

Wuest, S. E., Peter, R., & Niklaus, P. A. (2021). Ecological and evolutionary approaches to improving crop variety mixtures. *Nature Ecology & Evolution*, 5(8), 1068–1077. <https://doi.org/10.1038/s41559-021-01497-x>

Zuk, M., & Stoehr, A. M. (2002). Immune defence and host life history. *The American Naturalist*, 160(S4), S9–S22. <https://doi.org/10.1086/342131>

## Appendix 1

### Can Biopesticides and Environmental Variation Solve the Insect Resistance Problem?

*Did you know that around 20% of crops get eaten and destroyed by insects during growth or in storage? As the worldwide population grows, protecting crops becomes increasingly important. Farmers rely heavily on pesticides, but pest populations are becoming harder to control as many insect species develop resistance.*

#### A Potential Solution: Biopesticides

Chemical pesticides are biochemically simple and typically only target one or a few specific genes in insects. Only a few genetic changes have to be made for resistance and the pesticides will stop working. A solution to slow down resistance evolution could be biopesticides, including microbial biopesticides such as bacteria, fungi or viruses. Biopesticides are more complex, and often target many genes in the insects affecting multiple life-history traits like immune defense, growth and reproduction. The more genetic changes required, the harder it becomes for insects to develop resistance. Life-history traits are also under strong selection, and can involve trade-offs: increasing one trait, like immunity, can reduce another trait, like growth or reproduction. These trade-offs can potentially slow down resistance development.

#### The Problem with Modern Agriculture

Modern day agriculture is very homogenous, involving the use of the same crops and pesticides over large parts of the landscape. For insects, this creates an easy environment in which their main challenge is to fight off pesticides. Environmental variation is important because of a concept called gene-by-environment interactions, which explains that the effect of certain genes depends on the environment. By changing the environment, we can also change which gene variants are more effective in certain situations, making it harder for insects to evolve resistance. It is important to find ways for farmers to increase environmental variation, preferably easy and practical for farmers to implement, so that pests have a harder time evolving resistance.

#### Testing Different Doses of Fungal Pesticides in Moth Larvae

This study explores the use of different doses of fungal biopesticides as a form of environmental variation, to see how environmental variation affects trade-offs between survival, size and development rate. When farmers spray the biopesticides, not every insect will be sprayed by the same doses, meaning that dose is an already naturally occurring variation. Surprisingly, most larvae survived and grew big quickly. This was likely caused by favorable and easy laboratory conditions, including constant temperature and humidity, optimal food and no other pathogens. In agricultural environments, even if they are very uniform there is still more environmental variation and stressors than in the lab.

Still, even if the biopesticide did not significantly affect larval survival, there was still evidence for gene-by-environment interactions in development rate. This suggests that environmental variation like dose variation can potentially be important in affecting resistance evolution. However, dose variation alone is likely not enough; instead the combination of many environmental factors can create stress on the larvae and induce trade-offs that could constrain the evolution of resistance.

#### Why this matters

The low effects of the biopesticide on mortality and other traits highlight the complexity of studying resistance evolution. However, these results still showed that biopesticides combined with environmental variation, like dose variation, can be a promising strategy to constrain insect resistance evolution. If the worldwide population continues to grow, food security will become an even bigger concern. Finding sustainable solutions to protect crops is more important than ever.

## Appendix 2

### Mathematical notation for the statistical model

The mathematical notation for the statistical model I used to estimate genetic parameters is written down below:

$y_i$	$\sim \text{Normal}(\mu_i, \sigma)$	[likelihood for standardized traits]
$\mu_i$	$= \alpha + \beta_{\text{dose}[i]} + u_{\text{sire}[i], \text{dose}[i]} + v_{\text{dam}[i]}$	[linear predictor]
$\alpha$	$\sim \text{Normal}(0, 0.1)$	[prior for intercept]
$\beta_{\text{dose}}$	$\sim \text{Normal}(0, 0.1)$	[prior for dose effects]
$v_{\text{dam}}$	$\sim \text{Normal}(0, \sigma_{\text{dam}})$	[random effect for dam]
$\sigma_{\text{dam}}$	$\sim \text{Exponential}(1)$	[prior for dam SD]
$u_{\text{sire}, \text{dose}}$	$= L_{\rho_{\text{sire}}} \cdot \text{diag}(\sigma_{\text{sire}}) \cdot z$	[non-centered random sire-by-dose effect]
$z$	$\sim \text{Normal}(0, 1)$	[standard normal for sire effects]
$\sigma_{\text{sire}}$	$\sim \text{Exponential}(1)$	[prior for sire SDs]
$L_{\rho_{\text{sire}}}$	$\sim \text{LKJ\_Cholesky}(1)$	[prior for correlation]
$\sigma$	$\sim \text{Exponential}(1)$	[residual standard deviation]

NB: subscript notation in the linear predictor indicates that I fit separate estimates for doses, for each sire and dose combination, and for each dam.