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Sub-lethal viral exposure and growth on drought stressed host plants changes resource allocation patterns and life history costs in the Speckled Wood butterfly, *Pararge aegeria*

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Highlights

- Direct cost of growth when resisting viral infection (i.e. reduced pupal mass)
- Larvae had higher performance on drought stressed plants (i.e. heavier pupae)
- Costs of drought and viral exposure were larval mass-dependent
- Large larvae buffer some costs of growth in response to drought and infection

Abstract

This study investigated the interactive effects of growth on drought stressed host plants and pathogen challenge with the baculovirus *Autographa californica nucleopolyhedrovirus* (AcMNPV) on survival and fitness-related traits using the Speckled Wood butterfly, *Pararge aegeria* (L.). Exposure to AcMNPV significantly reduced survival to pupation. For surviving larvae, sub-lethal infection significantly decreased daily mass acquisition rates and pupal mass. Growth on drought stressed plants increased daily mass acquisition rates resulting in heavier pupae, and increased resource allocation to adult reproduction. The interaction between host plant drought and viral exposure resulted in different resource allocation strategies, and thus different growth trajectories, between larvae. This in turn resulted in significantly different allometric relationships between larval mass (at inoculation) and both development time and investment in flight muscles. For larvae with relatively lighter masses there was a cost of resisting infection when growth occurred on drought stressed host plants, both within the larval stage (i.e. longer larval development times) and in the adult stage (i.e. lower investment in flight muscle mass). This multi-factor study highlights several potential mechanisms by which the complex interplay between low host plant nutritional quality due to drought, and pathogen exposure, may differentially influence the performance of *P. aegeria* individuals across multiple life stages.

Keywords: Baculovirus; immune defence; host plant quality; compensatory growth

Introduction

With global climate change emergent infectious diseases have risen significantly and climate warming has resulted in changes in the severity and prevalence of some infectious diseases, pests and pathogens (Daszak et al., 2000; Jones et al., 2008; Bebber, 2015; Shikano and Cory, 2015; Kolb et al., 2016; Ogden and Lindsay, 2016). In ectotherms, like herbivorous insects, climate change can impact host-pathogen dynamics both directly and indirectly. For example, rising temperatures can directly increase the likelihood of an insect herbivore encountering pathogens via increased feeding rates (Eldred and Reilly, 2014). Increasing temperature and carbon dioxide levels may also reduce insect immune functioning and thereby directly increase susceptibility to disease by decreasing host plant quality (increasing plant defensive traits and decreasing nitrogen content) thereby decreasing immune functioning (Shikano and Cory, 2015). Another, as yet, underexplored mechanism by which climate change may impact host-pathogen dynamics is via an increase in the rate and magnitude of climatic extremes such as drought (e.g. Kolb et al., 2016). Similar to the indirect effects of increasing temperature on host plant quality, growth on drought-stressed host plants also has the potential to decrease insect herbivore immune functioning via its negative effects on host plant quality. Host plant quality can indirectly affect insect-pathogen interactions via two main routes, altering the susceptibility of the insect to the pathogen, and/or changing the behaviour of the insect (Cory and Hoover, 2006; Shikano, 2017). For example, many phytochemicals, such as defensive allelochemicals, can modify the physiology and growth of insects by affecting their susceptibility to infection (Cory and Hoover, 2006; Shikano, 2017). Drought can induce changes in the chemical composition of plants, affecting the concentration and balance of nutrients, altering levels of defensive allelochemicals and/or changing levels of volatile stress metabolites (Mattson and Haack, 1987). Drought-induced chemical changes in plants can influence interactions between insects and their pathogens directly, or indirectly by enhancing the insect's immune system (Mattson and Haack, 1987). For example, high levels of allelochemicals in drought stressed plants consumed by insects can inhibit the growth of ingested pathogens (Mattson and Haack, 1987). Additionally drought-

induced changes in nutrition can alter the insect's capacity to suppress pathogenic microorganisms (Mattson and Haack 1987).

Effective resistance to pathogens is often dependent on resource availability and resource quality, because resource availability and quality improves body condition and also immune system deployment (Shikano and Cory, 2015; McKay et al., 2016). From ecoimmunological studies and life history theory, there is good evidence that investment in pathogen defences comes at the expense of other physiological processes such as growth and reproduction (Stearns, 1992; Ricklefs and Wikelski, 2002; Schmid-Hempel, 2005; McKay et al., 2016). These life history trade-offs may be exacerbated by development on low quality host plants, as immune response and compensatory growth in response to low resource quality both use energy reserves from the same resource pool (Shikano and Cory 2015; McKay et al., 2016). Food restriction during development can alter fitness and immunity across both juvenile and adult life stages (McKay et al., 2016). Poor developmental nutrition has been shown to impact many adult fitness traits such as size (e.g. Nylin and Gotthard, 1998; Gibbs et al., 2012), reproductive success (e.g. McGraw et al., 2007; Gibbs et al., 2012; Schwenke et al., 2016) and adult lifespan and adult phenoloxidase activity (McKay e al., 2016). To further complicate matters, however, compensatory growth responses are not always predictable and depend on various factors such as species (e.g. Hayward and Wang, 2001; Zhu et al., 2001; but see Karl et al., 2013), social environment (Maclean and Metcalfe, 2001; Metcalfe and Monaghan, 2001), and physiological factors such as internal state and age (e.g. Nicieza et al., 1997). Mangel and Munch (2005) identified a range of possible compensatory responses such as; i) faster than normal growth immediately following the end of the deprivation period, ii) faster than normal growth at some time later (long term compensation) and iii) faster than normal growth and over-taking control individuals that continue to grow normally. As such there is unlikely to be a simple easily predictable response, and just knowing that immune functioning increases with resource quality may not be sufficient to determine the response of different species, or even different individuals within a species, to the interacting effects of food quality and pathogen exposure (Shikano and Cory, 2015).

As such there is a need for more complex, multi-factor, studies to examine the impact of ecologically relevant pathogen species on host survival and other fitness traits under poor nutritional growth conditions (Shikano and Cory, 2015).

Using the model pathogen, the baculovirus *Autographa californica multinucleocapsid nucleopolyhedrovirus* (AcMNPV), we examined the interactive effects of growth on drought stressed host plants and sub-lethal pathogen challenge on survival and fitness-related traits using the Speckled Wood butterfly, *Pararge aegeria* (L.). Baculoviruses are highly pathogenic obligate killers that infect the larval stage via ingestion of contaminated leaf material (Cory and Myers, 2003; Harrison and Hoover, 2012). AcMNPV has a broad host range, infecting at least 15 lepidopteran families (Cory and Myers, 2003; Shikano and Cory, 2015). Sub-lethal baculovirus infections have been shown to incur fitness costs to their hosts such as reduced pupal weight and prolonged larval development time (Milks et al., 1998; Shikano and Cory, 2015) and also by delaying the onset of egg laying (Shikano et al., 2016). Pathogens can directly affect insect wing development both positively (Ryabov et al., 2009) and negatively (Wülker, 1985; de Miranda and Genersch, 2010) and may also have indirect effects through resource trade-offs during development between immune defence and investment in flight morphological traits (Zuk and Stoehr, 2002). In Monarch butterflies, infection with pathogens can directly reduce flight ability, but this however, was not shown to be related to changes in wing morphological traits (Bradley and Altizer, 2005). Little is known about the effects of sub-lethal baculovirus infection on wing development and flight morphological traits. To date most studies have only crudely quantified effects by measuring wing deformities (e.g. Milks, 1997; but see Hesketh et al., 2012).

Pararge aegeria is one of the six most drought-sensitive butterfly species in the UK, whose populations could, under Global Climate Models of 'business-as-usual' emissions, suffer widespread extinctions by 2050 (Oliver et al., 2015). This butterfly species has been extensively researched and used as a model system for studies of insect ecology and life history evolution (e.g. Aalberg Haugen and Gotthard, 2014), including single factor studies examining life history trade-offs in response to

drought (Gibbs et al., 2012; Vande Velde et al., 2013) and viral infection with AcMNPV (Gibbs et al., 2010a; Hesketh et al., 2012). Little is known about the full range of pathogens *P. aegeria* encounter in nature, or the impact of known sub-lethal infections with bacteria (e.g. *Wolbachia*, Carter et al. 2013) or viruses (e.g. Rhabdoviruses, Longdon et al., 2017) on life history traits in natural populations. Previous laboratory studies on *P. aegeria* have shown that infection with AcMNPV reduces survival (Gibbs et al., 2010a; Hesketh et al., 2012), but has no observable sub-lethal direct effects on larval development time, pupal mass or adult wing morphology when larvae are growing under conditions of good resource availability and quality (Gibbs et al., 2010a; Hesketh et al., 2012). Drought stressed host plants have been shown to have reduced quality for *P. aegeria* larvae, with lower levels of foliar nitrogen, carbon and water available for consumption (Talloen et al., 2004), affecting adult mass, survival and investment in flight morphological traits (Talloen et al., 2004; Gibbs et al., 2012). *Pararge aegeria* is therefore an ideal model system with which to examine the impact of complex interactions between environmental factors on survival and fitness related traits.

Methods

Study species, Pararge aegeria

Along the pure capital breeding to pure income breeding continuum, *P. aegeria* sits closest to the capital breeding end of the spectrum and females of this species mostly rely on nitrogenous resources acquired during the larval stage for egg production (Bauerfiend and Fischer, 2008). There is little opportunity for females to obtain more nitrogenous resources for reproduction through adult feeding (Karlsson, 1994; Vande Velde and Van Dyck, 2013) or nuptial gifts (Svärd and Wiklund, 1989). During nectar and honeydew feeding, adults imbibe a dilute aqueous solution of mainly sugar with some lipids and minute quantities of amino acids (Jervis et al., 2008). Therefore, through adult feeding *P. aegeria* may gain extra carbohydrate and lipid resources to fuel somatic maintenance and flight (cf. Jervis et al., 2008; Vande Velde and Van Dyck, 2013). A sexual size dimorphism in size

occurs in *P. aegeria* (Sibly et al., 1997), and selection for large female size (and hence high fecundity) appears to be more important for fitness than selection for large male size (Gotthard et al., 1994; Leimar et al., 1994).

Baculovirus production

A plaque-purified variant of AcMNPV designated C6 (Possee, 1986) was propagated in *Spodoptera frugiperdu cells* (IPLB-Sf-21) (Vaughn et al., 1977). A stock of AcMNPV was obtained as described in Gibbs et al., (2010a). In brief, a stock of AcMNPV was obtained by dosing 3rd instar *Trichoplusia ni* (Hübner) larvae. Viral inoculum was added to small plugs of artificial diet and larvae were maintained individually and allowed to feed overnight. Once the entire plug of diet was consumed, larvae were incubated in individual pots containing artificial diet until death due to virus. Viral cadavers were collected, macerated and the resulting suspensions filtered through sterile muslin to remove large particulate matter. Viral material was then purified using density gradient centrifugation. The concentration of occlusion bodies was estimated by counting three times in an improved Neubauer haemocytometer at magnification 400x (<10% error in counts) (cf. Hesketh et al., 2012). Dilution of the stock suspension was done in sterile distilled water to achieve a final concentration of 1×10^6 OBs μl^{-1} for use in experiments. Previous studies (using the same methodology as described for this current study) with the model system, AcMNPV verified that when exposed to 1×10^6 OBs ml^{-1} at 21 days of larval development, when growth occurs on potted host plants of Rough-stalked Meadow grass, *Poa trivialis* (L.), 78% of *P. aegeria* survived to eclose as an adult (Gibbs et al., 2010a). As such, this concentration of AcMNPV was used to ensure that we could examine the sub lethal effects of viral infection for the majority of our experimental larvae (cf. Gibbs et al., 2010a; Hesketh et al., 2012).

Host plant treatment

Potted host plants (9 cm diameter, 0.36L pots) of Rough-stalked Meadow grass, *Poa trivialis* (L.) were grown from seed under standard conditions in a greenhouse (24°C, 12:12 L:D photoperiod). At maturation (40 days after germination) the plants were randomly assigned to one of two treatment groups: control or drought stressed. Control plants were watered daily both prior to the start of the experiment (i.e. 20 days before larval hatching) and throughout the experimental period (i.e. during larval development). Control plants were watered daily to prevent their soil from drying out (and the plant from wilting), but the soil was never oversaturated with water. Drought-stressed plants were deprived of water for 20 days prior to the start of the experiment and then were subsequently only provided with water once every 6 days throughout the experimental period. By only watering the plants every 6 days, we ensured that the plants remained alive (and had leaves available for larval consumption) but experienced moderate drought stress throughout the experimental period (after Talloen et al., 2004; Gibbs et al., 2012).

Larval life history

Pre-viral inoculation

Our experimental animals were derived from an outbred laboratory stock population, which originated from Belgium. Newly hatched F_3 generation larvae from this population were placed individually on labelled enclosed potted host plants of *P. trivialis* (either control or drought stressed) and reared under a direct development regime (LD 16:8 h photoperiod at 18°C) in a climate room. As a higher mortality was expected with drought stress (see Talloen et al., 2004; Gibbs et al., 2012), a total of 60 and 114 larvae were assigned to the control and drought stress treatment groups respectively. All of the larvae used in this experiment shared the same hatching date. Larval development and survival was recorded for each individual up to 21 days. At 21 days of larval development, all larvae were removed from their host plants, weighed (Ohaus Explorer balance; accuracy: ± 0.1 mg) and placed individually into labelled plastic pots lined with filter paper moistened with water. Larvae were then left for 24h without food to ensure consumption of

inoculated *P. trivialis* leaves (see below). Weighing larvae prior to starvation and pre-viral inoculation allowed us to compare larval growth pre- and post- inoculation, and enabled us to examine (during analyses) potential differences in response due to variation in larval mass at inoculation (cf. Gibbs et al., 2010a).

Post-viral inoculation

After 24h without food, half of the larvae from each host plant treatment group were assigned to one of two treatment groups: control or exposed to AcMNPV. Each larva in the control treatment group was given 3 x 1cm pieces of *P. trivialis* leaves inoculated with 1 microlitre of sterile water. Each larva in the infected treatment group was given 3 x 1cm pieces of *P. trivialis* leaves inoculated with 1 microlitre of 1×10^6 OBs ml^{-1} suspension of AcMNPV. All larvae were left for 24h to consume all of the *P. trivialis* leaves (after Gibbs et al., 2010a; Hesketh et al., 2012). After this inoculation period, each larva was returned to its own enclosed potted host plant of *P. trivialis*. Each individual was monitored daily up to pupation. Larval development time was recorded as the number of days from viral inoculation to pupation. Larval daily mass acquisition rates were calculated as (pupal mass – mass at inoculation)/larval development time (after Hesketh et al., 2012). On the day after pupation (once the pupal case had hardened) each pupa was weighed (Ohaus Explorer balance; accuracy: ± 0.1 mg) and placed into an individual container until eclosion as an adult. Survival to pupation and survival to eclosion as an adult were recorded for each individual. The experiment ended when all surviving larvae eclosed as an adult.

On the day of eclosion each adult was sexed. Butterflies were killed within 24 hours of emergence, after their wings had fully hardened, by placing them in a -20 ˚C freezer. Fore- and hindwings were carefully removed from the thorax. The body of each individual was dried for 24 h at 60 ˚C, and then weighed (Ohaus Explorer balance; accuracy: ± 0.1 mg). The thorax was then carefully removed and weighed, and used as a measure of investment in flight muscle mass (after Hughes et

al., 2003). The abdomen was weighed and used as a measure of investment in reproduction (after Karlsson, 1994).

Statistical analyses

Larval mass pre-inoculation, larval development time post-inoculation, larval daily mass acquisition rates, pupal mass, thorax mass and abdomen mass were each analysed by means of a General Linear Model (glm). Fixed factors were host plant treatment group and virus treatment group. Larval mass at inoculation was used as a continuous explanatory variable. Larvae that survived and developed to the pupal stage could be sexed, and thus sex was also added as a fixed effect to the models for larval development time, larval daily mass acquisition, pupal mass, thorax mass and abdomen mass. As such, for each of the post-inoculation life history traits measured, only animals that survived to pupation were included in the analyses. To take account of allometry effects, total dry mass was included as a covariate when analysing investment in thorax mass (i.e. flight muscle mass) and abdomen mass (i.e. investment in reproduction).

Survival to pupation and survival to eclosion (0 = dead, 1 = alive) were analysed using a generalized linear model with a logit link function. Fixed factors were host plant treatment group and virus treatment group. Larval mass at inoculation was used as a covariate. Offspring sex was not included in these models as offspring that did not survive could not be sexed. A Pearson chi-square test was used to compare the sex ratio of surviving adults across our 4 treatment groups (Control plant/Control virus vs. Control plant/exposed to virus vs. Drought plant/Control virus vs. Drought plant/exposed to virus).

All final models only included significant interactions. The continuous explanatory variable, larval mass at inoculation, and the covariate total dry mass, were only included in final models when significant. Analyses were performed in R 3.2.1 (package lme4; http://lib.stat.cmu.edu/R/CRAN/). Significances for Restriction Maximum Likelihood (REML) constructed models in R are estimated by means of t_{df} -values (glm) and z-values (glm with logit link function). The sign of either the t- or z-

values is indicative of the relationship between the effect and the dependent variable (i.e. positive or negative). All mean values are presented in the text with ± Standard Error (SE). Where relevant post-hoc analyses were performed to establish the significance of a factor level with respect to the other factor levels. Post-hoc analyses were done with the R package multcomp (using Tukey contrasts). Interaction effects between all of the explanatory variables were tested, including three-way interactions. Using a backward elimination procedure, larval mass at inoculation, total dry mass and interaction effects were only included in the final model (and hence presented in the text) when significant (i.e. $P < 0.05$). Residuals were examined for linearity and normality by inspecting normal probability plots and histograms of the residuals and by plotting the residuals versus the predicted values.

Results

Larval mass at inoculation (mg)

There were no significant differences in the mass of larvae at inoculation across virus treatment groups ($t_{1,142} = -0.25$, $P > 0.05$) or across host plant ($t_{1,142} = -0.36$, $P > 0.05$).

Larval development time (days)

There was an effect of sex on larval development time ($t_{1,144} = -3.77$, $P < 0.001$, Suppl. file S2). Based on post-hoc comparisons, male larvae had shorter development times than female larvae (Mean males = 14.61 ± 1.04, Females = 16.77 ± 0.73; $z = -3.77$, $P < 0.001$). There was a significant 3-way interaction effect between larval mass at inoculation, host plant treatment group and virus treatment group ($t_{1,144} = -3.70$, $P < 0.001$, Suppl. File S2). This means that although larval mass at inoculation has a significant effect on larval development time, the relationship between these two variables is fully dependent on the precise combination of host plant treatment and viral treatment.

That is, the slope of the regression line between mass and development time is dependent on the interaction between host plant and viral treatment. In particular, the slope for larvae exposed to a virus on control plants appeared the steepest. Thus, relatively speaking, larvae that were heavier at the time of inoculation had longer development times when exposed to a virus on control plants than those for the other 3 combinations (Figure 1). Likewise, at the opposite end of the larval mass at inoculation continuum, where larvae were relatively lighter in mass at inoculation, larvae on control plants exposed to a virus have the shortest development time (followed by larvae on drought stressed plants not exposed to a virus, larvae on drought stressed plants exposed to a virus, and larvae on control plants not exposed to a virus, which have the longest development time; Figure 1).

As detailed in the methods, all of the larvae used in this experiment shared the same hatching date and were weighed at a single time point at 21 days of development, prior to being assigned to a viral treatment group. Masses at this time point were observed to range between 2.4 and 83.7mg. From Figure 1, it can be seen that the two heaviest larvae at inoculation were randomly assigned to the control host plant/exposed to virus treatment group. We confirmed the validity of including these data by testing with and without these two data points. Although the precise values of the slopes change, neither the patterns nor the statistical significance was affected (Suppl. File S1, Figure S1). The data for these two "heavy" larvae were therefore included in these analyses as they appeared to be biologically meaningful (and see below for analyses of dry thorax mass).

Larval daily mass acquisition rates (mg/day)

There was a significant difference across viral treatments in larval daily mass acquisition rates ($t_{1,143}$ = -2.14, P < 0.05, Suppl. File S2). Based on post-hoc comparisons, larvae exposed to a virus had lower daily mass acquisition rates than unexposed larvae (Mean unexposed larvae = 7.81 ± 0.18, mean for exposed larvae = 7.32 ± 0.26; z= -2.27, P < 0.05). There was a significant difference across host plant treatments in larval daily mass acquisition rates ($t_{1,143}$ = 3.86, P < 0.001, Suppl. File

S2). Based on post-hoc comparisons, larvae developing on drought stressed host plants had higher daily mass acquisition rates than larvae developing on control host plants (Mean control plants = 6.91 ± 0.22, Drought-stressed plants = 7.90 ± 0.27; z = 3.86, P < 0.001). Males and females did not differ in their mass acquisition rates ($t_{1,143}$ = -1.53, P > 0.05; Suppl. File S2).

Survival to pupation (%)

There was a significant effect of viral treatment on survival to pupation ($z_{1,173}$ = -2.43, P < 0.05). Based on post-hoc comparisons, larvae that were exposed to AcMNPV had lower rates of survival to pupation (where 0 = all dead, and 1 = all alive; Mean for larvae not exposed to AcMNPV = 0.97 ± 0.03, mean for larvae exposed to AcMNPV = 0.87 ± 0.04; z = -2.43, P < 0.05). Previous work on *P. aegeria* has shown that larvae that die of a baculovirus infection (presence of OBs confirmed by staining with Giemsa solution) have a liquefied sack appearance and a hanging stance but remain intact (Hesketh et al. 2012). There was no observed viral mortality in the control (unexposed) group, in contrast to the viral exposure treatment group where baculovirus mortality in larvae was observed with typical baculovirus liquefied sack appearance and hanging stance. We did not, however, carry out additional tests to confirm that the presence of OBs, and so cannot completely rule out other sources of mortality in these virally exposed larvae. There were no differences in survival to pupation across host plant treatment groups ($z_{1,173}$ = 1.01, P > 0.05). Larvae that had heavier masses at the time of inoculation had significantly higher survival rates to pupation ($z_{1,173}$ = -3.68, P < 0.001).

Pupal Mass (mg)

There was a significant effect of viral treatment on pupal mass ($t_{1,143}$ = -2.27, P < 0.05, Suppl. File S2). Based on post-hoc comparisons, individuals exposed to AcMNPV were lighter at pupation than unexposed individuals (Mean for individuals exposed to AcMNPV = 145.26 ± 2.88, Unexposed individuals = 148.56 ± 2.01; z = -2.27, P < 0.05). There was a significant effect of host plant treatment

on pupal mass ($t_{1,143}$ = 3.70, P = 0.0003, Suppl. File S2). Based on post-hoc comparisons, larvae that developed on drought stressed host plants were heavier as pupae (Mean control plants = 142.23 ± 2.48 mg, Drought-stressed plants = 149.24 ± 3.03 mg; z = 3.70, P < 0.001). Males and females had significantly different pupal masses ($t_{1,143}$ = -11.44, P < 0.0001; Suppl. File S2). Based on post-hoc comparisons, males were lighter at pupation than females (Mean males = 135.58 ± 2.23 mg, Females = 158.78 ± 1.56 mg; $z_{1,143}$ = -11.44, P < 0.0001). Larvae that had heavy masses at the time of inoculation had significantly heavier pupae ($t_{1,143}$ = 2.97, P < 0.01, Suppl. File S2).

Survival to eclosion

There were no differences in survival rates to eclosion across virus treatment groups ($z_{1,173}$ = -1.27, P > 0.05) or host plant treatment groups ($z_{1,173}$ = 0.80, P > 0.05). Larvae that had heavy masses at the time of inoculation had significantly higher survival rates to eclosion ($z_{1,173}$ = 4.02, P < 0.0001). There were no differences in the number of males and females that survived to eclosion across treatment groups (χ^2 = 0.22, P > 0.05).

Resource allocation to flight muscle

Body mass scaled significantly with flight muscle mass. Adults with heavier body masses had higher resource allocation to flight muscle mass than adults with smaller body masses ($t_{1,144}$ = 8.06, P < 0.0001, Suppl. File S2). However, the nature of the (allometric) scaling between larval mass at inoculation and the investment in flight muscles (i.e. thus adult thorax mass) is fully dependent upon the interaction between host plant treatment and virus treatment (i.e. a 3-way interaction as observed before for larval development time; $t_{1,144}$ = 2.59, P < 0.05; Figure 2 and Suppl. File S1, Figure S2). For example, larvae with relatively lighter masses at inoculation, larvae exposed to a virus on control host plants allocated relatively more resources to thorax mass, followed by larvae not exposed to a virus on drought stressed plants, larvae exposed to a virus on drought stressed plants

and, larvae not exposed to a virus on control plant, which allocate the fewest resources to thorax mass (Figure 2).

At the opposite end of the larval mass continuum, where larvae are relatively heavier at inoculation, larvae exposed to a virus on control plants invested relatively fewer resources to thorax mass than the other 3 treatment groups (Figure 2). As before, the slope for larvae exposed to a virus on control plants appeared the steepest. These results for larvae with relatively heavier masses should, however, should be considered with some caution (Suppl. File S1, Figure S2).

Resource allocation to reproduction

There was no effect of virus infection on resource allocation to reproduction ($t_{1,144}$ = -1.16, P > 0.05, Suppl. File S2). Based on post-hoc comparisons, larvae reared on drought stressed host plants had a higher relative resource allocation to their abdomens, and hence a higher resource allocation to reproduction (Mean control plants = 8.1 ± 0.62, Drought-stressed plants = 9.3 ± 0.76; z = 2.03, P < 0.05). Based on post-hoc comparisons, males had lower relative resource allocation to reproduction than females (Mean males = 4.96 ± 0.32, Females = 12.73 ± 0.23; z = -6.23, P < 0.0001). There was also a significant relationship between dry total body mass and dry abdomen mass, with large adults having higher resource allocation to reproduction than small adults ($t_{1,144}$ = 23.93, P < 0.0001, Suppl. File S2).

Discussion

Previous studies on *P. aegeria* have shown that infection with AcMNPV reduces survival (Gibbs et al., 2010a; Hesketh et al., 2012), but has no observable sub-lethal effects on larval development time or pupal mass when larvae are growing under conditions of good resource availability and quality (Gibbs et al., 2010a). Prior to exposure to AcMNPV there were no differences in larval mass across viral treatment groups. Following exposure to AcMNPV, we observed that *P. aegeria* larvae had

lower daily mass acquisition rates, lower survival to pupation and smaller pupal masses, suggesting that there is a direct cost of growth when resisting viral infection in the larval stage.

Contrary to the findings of previous studies on *P. aegeria* (Gibbs et al., 2012; Talloen et al., 2004), larvae reared on drought stressed host plants had heavier pupal masses and there was no effect on survival. In addition, we also found that larvae that developed on drought stressed host plants had higher daily mass acquisition rates and higher resource allocation rates to reproduction. These differences may represent population-specific variation in growth responses on drought stressed hosts plants, but may also be due to differences in experimental design. For example, it is important to note that in the present study these responses were only observed after a short period of food deprivation prior to inoculation with AcMNPV. Before food deprivation and virus challenge, there were no differences in larval mass across host plant treatment groups (i.e. larval mass at inoculation). This suggests a potential compensatory response following the end of the food deprivation period, with faster than normal growth resulting in individuals developing on drought stressed host plants over-taking control (host plant) individuals that continue to grow normally. This resulted in larvae reared on drought stressed host plants having heavier masses at pupation. This is in accordance with previous studies on other temperate organisms that shorten their development after exposure to a simulated increase in time constraints (Johnson et al., 2012 and references therein) and costs of compensatory growth have been found in other invertebrate species (e.g. Damselfly, De Block and Stocks, 2004; Stoks et al., 2006; Ladybird, Dimitriew and Rowe, 2011; butterfly, Lee et al., 2015). Changes in insect behaviour such as compensatory feeding and/or higher feeding rates may incur a cost a cost in nature, particularly if it promotes contact with pathogen reservoirs and increases the likelihood of (re-)infection (Cory and Hoover, 2006 and references therein). Many phytochemicals, especially defensive allelochemicals, can modify the physiology and growth of insects by affecting their susceptibility to infection (Cory and Hoover, 2006; Shikano, 2017). We cannot rule out the possibility that changes in plant defence chemicals may have

improved the performance of *P. aegeria* on drought stressed plants, but further studies would be required to test this hypothesis.

Previous studies have shown that negative effects of infection are often weaker in older, or larger animals, with larger or older larvae being more resistant (Stairs, 1965; Evans, 1981; Teakle et al., 1986; Kirkpatrick et al., 1998; Grove and Hoover, 2007; Gupta et al., 2007). It is well recorded that lepidopteran larvae, including *P. aegeria*, can demonstrate developmental resistance to baculovirus infection, and this is often attributed to increasing body weight during larval development (e.g. Evans, 1981; and for *P. aegeria*, Gibbs et al., 2010a). In accordance with these previous studies, we found that large larvae at inoculation had heavier pupal masses and higher survival to both pupation and eclosion. These data suggest that large *P. aegeria* larvae may be able to buffer at least some of the costs associated with growth in response to both drought and infection with AcMNPV. *Pararge aegeria* that hatch from large eggs tend to be larger larvae that have improved performance when growth occurs on low quality host plants (Gibbs et al., 2010b), and when larvae are exposed to sub-lethal viral infection (Gibbs et al., 2010a). In nature, therefore, maternal resource allocation to egg size (and hence transgenerational maternal effects) may play an important role in determining the capacity by which *P. aegeria* can cope with changes in host plant quality and disease prevalence expected with continued climate change. But, variation in food quality that influences egg size does not necessarily always affect offspring immunity (Shikano et al., 2016; Olson et al., 2017). Further studies would therefore be required to determine whether egg size plays a role in offspring immunity in *P. aegeria*. Importantly though, our results also show that drought stressed host plants and viral exposure affect the scaling relationships between larval mass and development time, as well as between larval mass and investment in flight muscles. Such slope differences are apparent when investigating larvae at the ends of the mass spectrum (i.e. light or heavy). For example, compared to larvae not exposed to AcMNPV, larvae with relatively lighter masses at the time of inoculation on control plants had shorter development times and higher investment in flight muscle mass when they were exposed to AcMNPV. Compared to larvae not

exposed to AcMNPV, larvae with relatively lighter masses at the time of inoculation reared on drought stressed host plants and exposed to AcMNPV, had longer development times and lower investment in flight muscle mass. This suggests that at the time of exposure to AcMNPV, larvae with masses at the lower end of the body mass continuum make different growth and resource allocation decisions based on the quality of food available, which becomes apparent in the significantly different allometric relationships observed. Under good food quality, no drought conditions, larvae with relatively smaller masses have fast development and higher investment in adult flight. Under low food quality, drought conditions, larvae with relatively smaller masses have longer development times and lower investment in adult flight. For larvae with relatively smaller masses there is therefore a cost of resisting infection when growth occurs on drought stressed plants, both within the larval stage (e.g. longer development times) and also later in the adult stage (e.g. reduced investment in thorax flight muscle mass). Further work is required, however, to fully disentangle these complex mass-dependent growth responses under different host plant and viral exposure treatments. In nature, decreased investment in adult flight has the potential to reduce the ability of individuals to move out of drought-stricken areas and/or areas with higher pathogen prevalence, and may also potentially negatively affect foraging, oviposition and mate-location behaviour too. This also requires further investigation, however, because variation in investment in flight morphological traits does not always translate into a direct reduction in locomotor capacity in this species (Lailvaux et al., 2016). Furthermore, additional work would be required to determine the relevance of these results to natural *P. aegeria*-pathogen systems.

Compared to females, male *P. aegeria* were observed to have shorter development times, lower daily mass acquisition rates, smaller pupal masses, reduced resource allocation to reproduction and increased resource allocation to flight. We found no evidence for sex-specific life history or survival differences in response to either drought or infection with AcMNPV. Differences across males and females in growth and resource allocation patterns reflect differences in life history. Females invest in slow growth, large size and have increased relative resource allocation to

reproduction (Gotthard et al., 1994; Leimar et al., 1994). Males and females also differ in their resource allocation patterns to flight morphological traits (e.g. thoracic mass, and hence flight muscle mass), with males investing in fast acceleration take-off flights used during territorial fights (Berwaerts et al., 2002; Berwaerts et al., 2008; Vande Velde and Van Dyck, 2013).

Our study highlights several potential mechanisms by which the complex interplay between low nutritional quality due to drought, and pathogen exposure, may differentially influence the performance of *P. aegeria* individuals across multiple life stages. Furthermore, our study demonstrates complex mass-dependent costs of growth on drought stressed host plants and when resisting a viral infection, resulting in a resource allocation trade-off in investment to future adult flight. This may reduce the ability of individuals to move out of drought-stricken areas and/or areas with higher pathogen prevalence. Potentially this reduction in dispersal capacity may have a greater impact on populations in fragmented landscapes (e.g. Gibbs et al., 2012). Our data indicate that *P. aegeria* populations may be susceptible to a combined increase in the frequency of summer drought and disease prevalence, both of which are expected with continued climate change.

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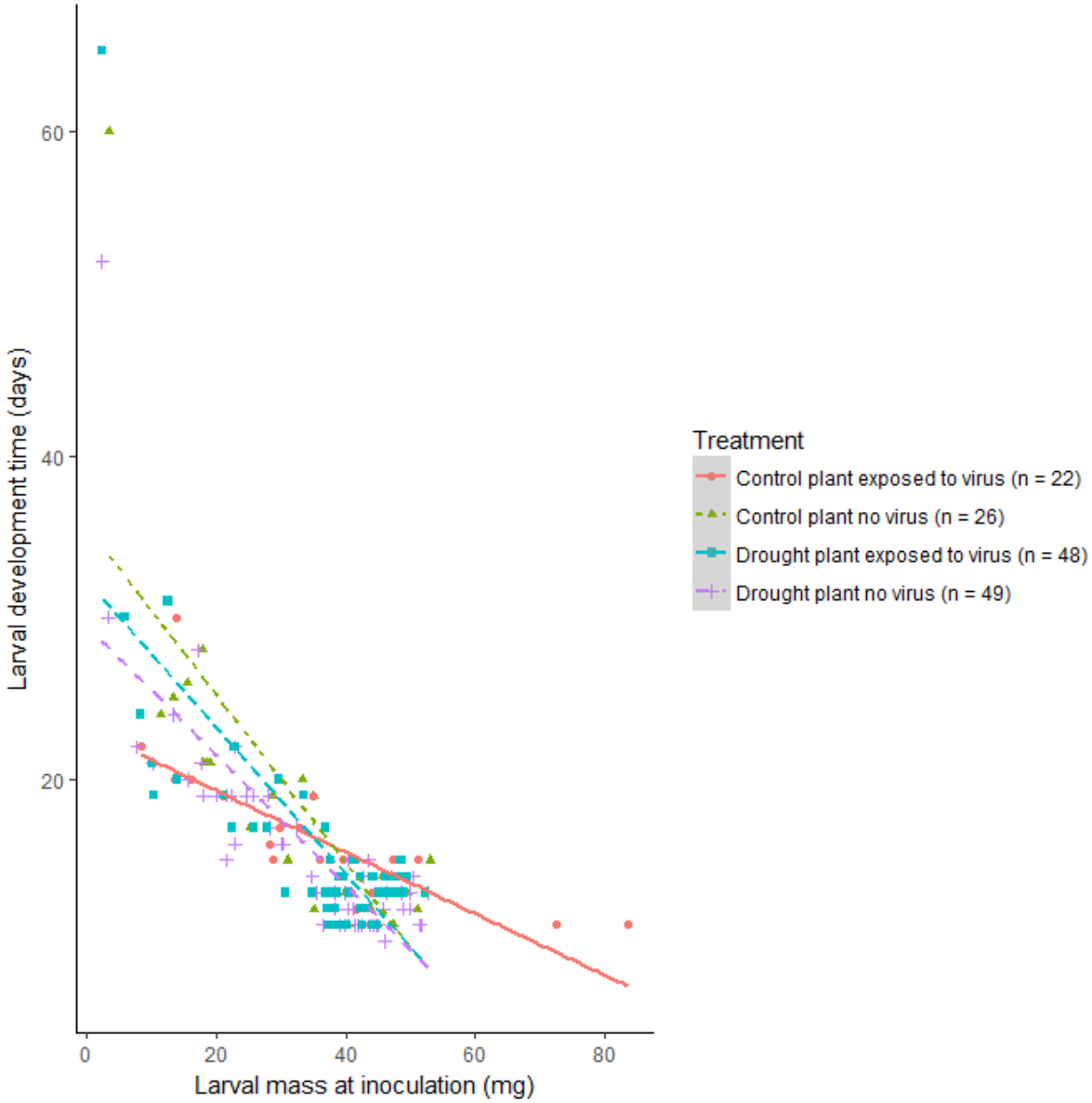
Figure 1. A 3-way interaction effect between larval mass at inoculation (mg), host plant treatment group (control plants vs. drought stressed plants) and viral treatment group (control, not exposed to a virus vs. exposed to a virus) on larval development time (in days).

Figure 2. A 3-way interaction effect between larval mass at inoculation (mg), host plant treatment group (control plants vs. drought stressed plants) and viral treatment group (control, not exposed to a virus vs. exposed to a virus) on adult thorax mass (mg).

Figure 1.



		Response to treatment	
Life stage	**Trait**	**Viral exposure**	**Drought stressed host plants**
	Larval development time	Viral infection x host plant treatment x larval mass interaction effect: The response to drought and viral exposure depended on the size of the larva	
	Larval daily mass acquisition rates	↓	↑
	Survival to pupation	↓	No effect
	Pupal mass	↓	↑
	Survival to eclosion	No effect	No effect
	Investment to reproduction	No effect	↑
	Investment to flight (thorax mass)	Viral infection x host plant treatment x larval mass interaction effect: The response to drought and viral exposure depended on the size of the larva	