Transgenerational effects of food quantity and quality on disease resistance in the western tent caterpillar, *Malacosoma californicum pluviale*

by

Grant Link Olson

B.Sc., Eckerd College, 2008

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY

Summer 2014

Approval

Name: **Grant Link Olson**

Degree: **Master of Science**

Title: ***Transgenerational effects of food quantity and quality on disease resistance in the western tent caterpillar, Malacosoma californicum pluviale.***

Examining Committee: **Chair:** Dr. Francis Law
Professor

Dr. Jenny Cory
Senior Supervisor
Professor

Dr. Bernard Roitberg
Supervisor
Professor

Dr. Judith Myers
Supervisor
Professor Emerita
Zoology
University of British Columbia

Dr. Steve Perlman
External Examiner
Associate Professor
Biology
University of Victoria

Date Defended: July 25, 2014

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Abstract

Increasing population density may alter food quantity or quality. Dietary stressors can interact simultaneously and produce emergent fitness effects both intragenerationally and potentially transgenerationally, including changes in pathogen resistance. Western tent caterpillar (WTC) *Malacosoma californicum pluviale* populations undergo regular 6-11 year cycles; epizootics of nucleopolyhedrovirus (NPV) characterize population declines and may be triggered by density-related dietary changes. I tested the transgenerational interaction of three factors likely to be influenced in rising WTC populations. I manipulated foliage quantity, quality, and the presence of phylloplane bacteria provided to the parental generation and assessed NPV resistance and immunity in their offspring. Food limitation had strong impacts on life history traits of tent caterpillars. Somewhat unexpectedly, changes in foliage quality and ingestion of phylloplane bacteria had transgenerational effects on offspring, enhancing their NPV resistance and affecting expression of background pathogens. There was no evidence for increased disease susceptibility in offspring from combined parental stressors.

Keywords: Food quality; transgenerational effects; viral epizootic; immunity; disease resistance; forest Lepidoptera

I dedicate this thesis to my family, for their love and support throughout my life. Their encouragement helps to keep me going in the right direction. To my parents, Ben and Sylvia, thank you for bringing me into this world and providing a nurturing home. To my brother, Sky, thank you for your mentorship, advice, and friendship. I feel blessed to share this lifetime with you all.

Acknowledgements

Firstly, I would like to thank Dr. Jenny S. Cory for her role as senior supervisor. I couldn't have asked for a more supportive and caring supervisor. Jenny is devoted to her students and is one of the hardest working people I have ever met. I deeply enjoyed working with her throughout this degree and feel that I have learned a great deal from her mentorship.

I would like to thank my committee members, Dr. Judy Myers and Dr. Bernie Roitberg. Judy's willingness to share her experise in tent caterpillars and population cycles, as well as her practical nature and decisiveness, were much appreciated. Judy's (and Jenny's) help was also indispensible for the design, set-up and carrying out of experiments. I also thank Bernie for his guidance with experimental design, data analysis, and overall support and input in crafting this thesis.

Thank you to thank Sean Haley, Colin McCune, Vincent Fung, and Tristan Takaro for all your help with feeding caterpillars, counting egg masses, and performing moth dissections. A special thanks to Ikkei Shikano for acting as my mentor in the Cory Lab, helping me develop my experimental design skills and immunological/bioassay techniques. Thank you to Anna DiCarlo for her help with acquiring bacterial stocks and instructing me in microbiological techniques. To all the other professors, colleagues, volunteers, friends, and loved-ones for their help, support, and advice throughout this degree, thank you.

Finally, I would like to thank the sponsors that made this research possible: NSERC Discovery Grant to Jenny S. Cory, Simon Fraser University Graduate Fellowships, H.R. McCarthy Graduate Bursary, and the Mutual Fire Insurance of BC Graduate Scholarship in Biological Sciences to Grant Olson, Simon Fraser University, and the University of British Columbia.

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Chapter 1.

Introduction

Transgenerational effects occur when the environment a parent experiences influences offspring phenotype and performance, apart from direct genetic transmission. These effects have been well documented across a broad range of taxa, including vertebrates, invertebrates, and plants (see reviews in Mousseau & Dingle, 1991; Bernardo, 1996; Rossiter, 1996; Mousseau & Fox, 1998; Molinier et al., 2006; Green, 2008). In invertebrates, transgenerational effects have become increasingly recognized as important factors that can affect fitness-related traits (Rossiter, 1991; Corkum et al., 1997; Mitchell and Read, 2005; Freitak et al., 2009a; Sadd & Schmid-Hempel, 2009; Frost et al., 2010; Roth et al., 2010; Zanchi et al., 2012) and population dynamics (Mousseau and Fox, 1998; Benton et al., 2001; Hunter, 2002; Plaistow et al., 2006; Benton et al., 2008). Mechanistically, environmental conditions interact with genotype to influence the expression of phenotypic traits and condition; this in turn affects resource provisioning to offspring (Leather, 1985; Benton et al., 2005; Mitchell & Read, 2005; Sadd & Schmid-Hempel, 2007; Boots & Roberts, 2012) or gene expression (Freitak, 2009a,b). Maternal effects are the most commonly cited type of transgenerational effect, although recent studies have found evidence for paternal effects in invertebrates as well (Bonduriansky & Head, 2007; Roth et al., 2010; Triggs & Knell, 2012). Transgenerational effects in invertebrates have been found to persist for several generations in some cases (Benton et al. 2005; Plaistow et al., 2006; Benton et al., 2008; Hafer et al., 2011).

Although the existence of transgenerational effects is widely accepted, debate continues as to their ecological and evolutionary importance (see reviews in Marshall & Uller, 2007; Badyaev & Uller, 2009; Bonduriansky et al., 2012; Uller et al., 2013). Of particular interest is the hypothesis that parental effects in invertebrates can induce transgenerational adaptive plasticity, i.e. that predictive environmental cues experienced by parents can stimulate phenotypic plasticity in progeny, thereby increasing offspring

fitness (Mousseau & Dingle, 1991; Rossiter, 1991; Fox & Mousseau, 1998). A number of invertebrate studies support this theory (Fox, 1997; Rotem et al, 2003; Kudo & Nakahira, 2005; Moret, 2006; Crean et al., 2013), although there is a healthy level of skepticism for this being a generality, as transgenerational effects can also have negative impacts on offspring fitness (see reviews in Bernardo, 1996; Rossiter, 1996; Mayhew, 2001). In these situations, it has been proposed that transgenerational effects may simply be pathways through which environmental variation is passed on or the result of physiological side effects (Fox & Czesak, 2000), though these explanations fail to address such issues as the conflicts between parental and offspring fitness and the level of environmental variability over time (Marshall & Uller, 2007; Räsänen & Kruuk, 2007; Hoyle & Ezard, 2012). The level of temporal environmental heterogeneity and relative "direction" of transgenerational effects may be particularly important when considering their potential adaptive value. As an example, a maternal effect is "positive" (Lande & Price, 1989) when large mothers lead to large offspring, but "negative" (Falconer, 1965; Janssen et al., 1988) when parent/offspring phenotypes are opposite in direction. While positive maternal effects are most advantageous in environments undergoing extreme shifts (facilitating rapid adaptation), negative maternal effects are predicted to maximize fitness in stable environments (slowing or reversing phenotypic changes), (Hoyle & Ezard, 2012). Therefore, it is likely that the nature and potential adaptive value of transgenerational effects is highly context dependent.

One way in which transgenerational effects may influence individuals and populations is through modulation of host susceptibility to pathogens. There is increasing evidence that susceptibility to invertebrate pathogens can be influenced transgenerationally through effects of parental diet, stress or previous pathogen exposure (Mitchell and Read, 2005; Sadd & Schmid-Hempel, 2009; Frost et al., 2010; Gibbs et al., 2010; Tidbury et al., 2011).

Pathogens, disease resistance, and immunity in invertebrates

Pathogens are ubiquitous and can cause high levels of mortality and disease across taxa. Their biology and impact has received considerable attention in human populations and for species of economic importance. The development of pathogens as biological control agents for insect pest species has been an area of intense focus for

decades (Steinhaus, 1956; Tinsley, 1979; Bedding et al., 1993; Lacey et al., 2001; Shah & Pell, 2003; Romeis et al., 2006; Hajek et al., 2007). In contrast, field studies investigating the ecological and evolutionary role of pathogens in insect populations and communities constitute a novel, emergent area (Altizer et al., 2000; Siva-Jothy, 2000; Cory & Myers, 2009; Vega et al., 2009).

A wide array of pathogens infects insects, with the most common groups being fungi, bacteria, viruses, and entomopathogenic nematodes, as well as the less common fungi-like oomycetes. Almost all insect orders are susceptible to entomopathogenic fungi and unlike most other entomopathogens they almost always initiate infection through the cuticle of the insect (Hajek and Leger, 1994). There are a number of ecologically important groups of entomopathogenic fungi, including the orders Hypocreales (*Metarhizium* spp. and *Beauveria* spp., both widely employed in biological control) (Roberts & St Leger, 2004; Meyling & Eilenberg, 2007) and Entomophthorales (Roberts, 1973; Pell et al., 2001).

Entomopathogenic bacteria are generally classified in two groups, spore-forming and non-spore-forming. Non-spore-forming bacteria are generally benign whereas spore-forming bacteria produce environmentally resistant spores designed to infect insect hosts (de Maagd et al., 2003). One of the most studied and exploited spore-forming bacterial species is *Bacillus thuringiensis* (Bt) (Aronson et al., 1986; de Maagd et al., 2003).

Insect viruses are diverse and infect an extensive range of hosts (Cory & Myers, 2003; Clem & Passarelli, 2013). The most well-known and best studied group of insect viruses are the baculoviruses (Baculoviridae). These viruses have been isolated from Lepidoptera, hymenopteran sawflies and Diptera. They produce environmentally robust proteinaceous occlusion bodies (OBs) that harbor one or more virions, depending on virus species. The infection process is initiated when larvae consume OBs present on food surfaces, triggering the release of virions that fuse with the midgut epithelial cells. In Lepidoptera, infection spreads from the midgut cells to other tissues in a budded form (a genetically similar but morphologically distinct form of the virus), eventually converting the larval body into millions of OBs. When the host dies, OBs are released into the

environment, contaminating plant surfaces and allowing the virus to perpetuate transmission (Cory and Myers, 2003).

The success of the virus and its ability to transmit to future hosts is, as with all pathogens, affected by the host's level of susceptibility and immunocompetence. Host resistance to infection may be related to selection pressures over evolutionary time or condition dependence based on environmental factors at the ecological scale (Roy & Kirchner, 2000; Cory & Myers, 2003; Beldomenico et al., 2008). Host plant can affect the midgut peritrophic matrix, which provides a physical barrier to viral infection (Plymale et al., 2008) and can influence sloughing of infected midgut cells, preventing the establishment and spread of the virus (Washburn et al., 1995; Hoover et al., 2000). The relationship between the insect immune system and baculovirus infection is still largely unknown, though recent evidence suggests that changes in gene expression post-infection (Berretta et al., 2013; Jakubowska et al., 2013) and melanisation of virus infected tracheal cells (Washburn et al., 1996; Trudeau et al., 2001; Sparks et al., 2008) are important factors.

The immune system of insects is reliant on innate immune mechanisms, which are triggered by infection and serve to destroy invading microbes. Pattern recognition proteins and immune signaling pathways can stimulate the insect immune system, leading to activation of the prophenoloxidase system, creation of differentiated haemocytes (which function to phagocytise and melanise/encapsulate infectious bodies) and production of antimicrobial peptides (Gillespie et al. 1997; Rowley & Powell, 2007; Jiang et al., 2010). The vertebrate immune system not only possesses innate functions, but is also characterised by a lymphocyte-based immune system capable of clonal expansion, memory, and specificity in response. This has been demonstrated not only within generations, but has also been shown to occur in a transgenerational manner, principally through the transfer of antibodies and pathogen resistance from mothers to offspring (Grindstaff et al. 2003; Grindstaff 2008). Although the immune system of invertebrates has been historically characterised by its supposed primitiveness and purely innate nature, emerging evidence suggests that invertebrates possess immune systems that may be functionally akin to vertebrates, having adaptive memory-like responses termed 'immune priming' (Moret & Siva-Jothy 2003; Korner & Schmid-

Hempel, 2004; Mowlds et al., 2008; Tate & Rudolf, 2012) that may show specificity in nature (Hoffman, 2003; Sadd & Schmid-Hempel, 2006; Roth et al., 2009). Although definitions vary, immune priming broadly refers to exposure to an immune insult (often a sublethal pathogen challenge) or elicitor (not necessarily pathogenic) increasing immunocompetence (with the expectation of increased resistance to future pathogen challenges). A number of recent invertebrate studies have also shown that these memory-like responses can be passed from parents to offspring through what is termed transgenerational immune priming (TGIP) (Little et al., 2003; Moret, 2006; Sadd & Schmid-Hempel, 2009; Freitak et al. 2009a,b; Tate & Rudolf, 2012). Both immune priming and TGIP involve mounting and maintaining an immune response, which is costly (Freitak et al. 2007; Sadd & Schmid-Hempel 2009). Resource availability or quality can play a role in modulating this cost (Rossiter, 1991; Ali, 1998; Lee et al., 2006; Shikano et al., 2010; Myers et al., 2011; Boots and Roberts, 2012).

Changes in resource availability and quality with herbivore density

Resource availability and quality are often linked to population density. Resource shortage is a common consequence of high population density among herbivorous insect defoliators (Lepidopterans: Diss et al., 1996; Carisey & Bauce, 2002; Abbot & Dwyer, 2007; Myers & Cory, 2013; Sawflies [Hymenoptera: Symphyta]: Piene et al., 2001; Lyytikäinen-Saarenmaa & Tomppo, 2002; Burnside et al., 2013; Coleopterans: Ferro, 1985; Elliot et al., 1996; Grimbacher et al., 2011). Changes in host plant quality are also linked with increasing herbivore population density (Underwood, 1999, 2010; Lynch et al., 2006), generally coming in the form of reduced foliage nutrient content or increases in the induction of chemical defenses (see reviews in Mayer 2004; Cory & Hoover, 2006; Schaller 2008; Fürstenberg-Hägg et al., 2013). Within a generation, variable resource availability or quality can affect invertebrate life history traits (Rossiter et al., 1988; Donegan & Lighthart, 1989; Boggs & Freeman, 2005; Sarfraz et al., 2013), immunity (Siva-Jothy & Thompson et al., 2002; Kapari et al., 2006; Haviola et al., 2007; Yang et al, 2007; Myers et al., 2011; Martemyanov et al., 2012), and disease resistance (Hunter & Schultz, 1993; Duffey et al., 1995; Cory et al., 1997; Hoover et al., 2000; Sarfraz et al., 2013; Cook et al., 2003). There is also emerging evidence for resource-influenced transgenerational effects on invertebrate life history traits (Rossiter, 1991; Frost et al., 2010; Valtonen et al., 2012; Saastamoinen et al., 2013), immunity (Boots &

Roberts, 2012; Triggs & Knell, 2012), and disease resistance (Gliwicz & Guisande 1992; Mitchell & Read, 2005; Frost et al., 2010; Boots & Roberts, 2012), though such phenomena are not well understood.

The consumption of non-pathogenic bacteria has been shown to have effects on insect immune expression, function and fitness traits in several studies, both within generations (Vodovar et al., 2005; Freitak et al., 2007) and transgenerationally (Freitak 2009a,b). Thus, changes in the (non-pathogenic) microbial community of the phyllosphere (defined as the aerial parts of plants) may be another way in which the quality of host plants may be connected with insect herbivore population density and disease. The phyllosphere is a habitat for numerous and abundant microorganisms, largely dominated by bacterial species (see reviews in Kinkel, 1997; Andrews & Harris, 2000; Lindow & Brandl, 2003; Meyer & Leveau, 2011; Vorholt, 2012). Communities and populations of leaf microbiota may change over time due to a variety of factors, including seasonality, nutrient availability, plant species, leaf surface properties, and microclimate (O'Brien & Lindow, 1989; Kinkel, 1997; Andrews & Harris, 2000; Kinkel et al., 2000; Lindow & Brandl, 2003). Despite the relative profusion of both insects and leaf microbiota residing in the phyllosphere, relatively little information exists as to what effects each group has on the other (Stadler & Müller, 2000) and such interactions are given little, if any, mention in otherwise comprehensive reviews considering leaf surface microbial population dynamics (Kinkel, 1997; Andrews & Harris, 2000; Lindow & Brandl, 2003; Meyer & Leveau, 2011). Though few in number, experiments investigating interactions between insect herbivores and leaf microbiota suggest that insect herbivory can increase both the abundance and diversity of leaf microbial populations (Stadler & Müller, 2000; Müller et al., 2003). The other side of the coin, whether leaf microbial communities can affect insect herbivores, is essentially unexplored.

Population cycles and pathogens

It is evident that changes to resource quantity and quality associated with increases in insect herbivore density can have important transgenerational effects on invertebrates. Transgenerational effects can provide a source of delayed density dependence or lag, which is a necessary prequisite for the existence of population cycles (May, 1973; Berryman, 1987). Lotka (1932) and Volterra's (1926) theories, along

with the work of Elton and Nicholson (1942) and Anderson and May (1981), highlighted the importance of exogenous mechanisms such as predation and parasitism in the cyclicity of animal populations. Probably the best-known groups that experience cyclic dynamics are small mammals and their predators (Lambin et al., 2000; Hanski et al., 2001; Huitu, 2007) and forest Lepidoptera (Myers, 1988; Myers & Cory, 2013). Many hypotheses have been put forward to explain what causes population cycles in forest Lepidoptera, including parasitoids (Berryman, 1996, 2002), predation (Klemola et al., 2009), pathogens (Dwyer & Elkinton, 1993; Myers, 2000; Cory & Myers, 2009), induced plant defenses (review in Nykänen & Koricheva, 2004; Kessler et al., 2012), transgenerational effects (Rossiter, 1994; Ginzburg & Taneyhill, 1994; Inchausti & Ginzburg, 2009), and changes in insect quality and immunocompetence (Haukioja, 2005; Kapari et al., 2006; Cory & Hoover, 2006; Reynolds et al., 2011).

Disease outbreaks have been implicated as important factors in the decline of populations in certain species of forest Lepidoptera that exhibit cyclic dynamics (Myers & Cory, 2013). In particular, epizootics of baculoviruses are associated with population crashes across a range of Lepidoptera (mainly in the Lymantriidae and Lasiocampidae), such as the gypsy moth, *Lymantria dispar* (Dwyer & Elkinton, 1993; Dwyer et al., 2000; McManus & Csóka, 2007; Martemyanov et al., 2012; Elderd et al., 2013; Alalouni et al, 2013), tussock moths, *Orgyia* spp. (Thompson et al., 1981; Richards et al., 1999; van Frankenhuyzen et al., 2002; Williams et al., 2011), and the western tent caterpillar, *Malacosoma californicum pluviale* (Myers, 2000; Cory & Myers, 2009; Myers et al., 2011).

Study system and overall questions

Population cycles of the western tent caterpillar (WTC) are particularly well-studied (Wellington, 1960, 1965; Myers, 2000; Cory & Myers, 2009). This system is ideal for testing concepts related to population cycles for a number of reasons: 1) the existence of an extensive population data set showing clear patterns relating to survival, fecundity, and disease, 2) a regular period of cycles (6 – 11 years) with one generation per year, and 3) females lay eggs in single masses and larvae are gregarious, facilitating the collection of fecundity-related and family-based/genetic data (Myers & Cory, 2013).

The WTC subspecies used in this study, *M. c. pluviale*, primarily ranges through much of the Pacific Northwest, though it is also distributed longitudinally across the North American continent in the Nearctic zone (Stehr & Cook, 1968; Fitzgerald, 1995). This species is polyphagous on a number of deciduous plants, including hawthorn (*Crataegus monogyna*), crab apple (*Malus diversifolia*), wild rose (*Rosa nutka*), and, chiefly, red alder (*Alnus rubra*) (Myers, 2000). As their name suggests, families of western tent caterpillars construct conspicuous silken tents on host plants from which they obtain shelter, a means to thermoregulate, and protection from predators (Fitzgerald, 1995).

In the early to mid stages of population increase, larval survival and moth fecundity are high (Myers, 2000). However, when density increases to near peak stages, larval survival begins a steep decline, followed by a decline in fecundity (Myers, 1988, 2000). High levels of disease beset peak populations of the WTC, primarily in the form of epizootics of the baculovirus *M. c. pluviale* nucleopolyhedrovirus (McplNPV) (Myers, 2000). These epizootics are associated with population crashes, leading to trough periods and lag effects of low fecundity for up to several years after the decline (Myers, 2000), possibly as a consequence of sublethal virus infection (Boots & Begon, 1994; Myers & Kukan, 1995; Cory & Myers, 2009).

Little is known as to how McplNPV persists in WTC populations during trough periods with low larval densities and no evidence of virus mortality. Environmental persistence of NPV in areas protected from UV can re-infect Lepidopteran larvae in a subsequent year (Rothman, 1997; Kukan, 1999; Fuller et al., 2012), but it is unclear how viruses can persist in the environment over successive years without evident infection. McplNPV in Lepidopteran populations could potentially persist by external contamination of the egg mass (Murray & Elkinton, 1989; Hugar et al., 1994; Kukan, 1999) or as sublethal or persistent infections, passed on vertically from parent to offspring (Burden et al., 2002, 2003, 2006; Cory & Myers, 2003). Stressors, such as other pathogens, host density, fluctuations in temperature/humidity, and diet have been proposed as the proximate triggers for virus activation into overt form (Fuxa et al., 1999; Cooper et al. 2003a,b; Il'inykh & Ul'yanova, 2005); however, no consistent trigger has been demonstrated.

In this thesis, I investigate the potential impacts that transgenerational, density-related, dietary factors have on WTC virus resistance, immunity, and life history traits. Specifically, I tested the effect of three factors (food quantity, herbivore-induced changes in foliage quality, and the presence of leaf bacteria) singly and in combination and whether they may interact to increase larval susceptibility to disease in offspring. The synergism of suboptimal dietary factors could provide an explanation for enhanced disease susceptibility in dense WTC populations that allows for the initiation of virus epizootics. While this thesis addresses a specific system and question, it is also intended as a test of general principles relating to how environmental factors can interact transgenerationally to produce trade-offs between life history traits and immunity/disease resistance.

1.A. References

Abbott, K. C., & Dwyer, G. (2007). Food limitation and insect outbreaks: complex dynamics in plant-herbivore models. *Journal of Animal Ecology*, *76*(5), 1004–14.

Alalouni, U., Schädler, M., & Brandl, R. (2013). Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. *Journal of Applied Entomology*, *137*(10), 721–738.

Ali, M. (1998). Influence of interspecific and intraspecific host plant variation on the susceptibility of *Heliothines* to a baculovirus. *Biological Control*, *12*(1), 42–49.

Altizer, S. M., Oberhauser, K. S., & Brower, L. P. (2000). Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecological Entomology*, *25*, 125–139.

Anderson, R. M., & May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *291*(1054), 451–524.

Andrews, J. H., & Harris, R. F. (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology*, *38*, 145–180.

Aronson, A. L., Beckman, W., & Dunn, P. (1986). *Bacillus thuringiensis* and related insect pathogens. *Microbiological Reviews*, *50*(1), 1–24.

Badyaev, A. V, & Uller, T. (2009). Parental effects in ecology and evolution: mechanisms, processes and implications. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1520), 1169–77.

Bedding, R. A., Akhurst, R. J., & Kaya, H. K. (Eds.). (1993). *Nematodes and the biological control of insect pests*. East Melbourne: CSIRO Publishing. 178 p.

Beldomenico, P. M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., & Begon, M. (2008). Poor condition and infection: a vicious circle in natural populations. *Proceedings. Biological Sciences / The Royal Society*, *275*(1644), 1753–9.

Benton, T. G., Plaistow, S. J., Beckerman, a P., Lapsley, C. T., & Littlejohns, S. (2005). Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society of B: Biological Sciences*, *272*(1570), 1351–6.

Benton, T. G., Ranta, E., Kaitala, V., & Beckerman, A. P. (2001). Maternal effects and the stability of population dynamics in noisy environments. *Journal of Animal Ecology*, *70*(4), 590–599.

Benton, T. G., St Clair, J. J. H., & Plaistow, S. J. (2008). Maternal effects mediated by maternal age: from life histories to population dynamics. *The Journal of Animal Ecology*, *77*(5), 1038–46.

Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist*, *36*, 83–105.

Berretta, M. F., Ferrelli, M. L., Salvador, R., & Sciocco, A. (2013). Baculovirus Gene Expression. In *Current Issues in Molecular Virology- Viral Genetics and Biotechnological Applications* (pp. 57–78).

Berryman, A. A. (1987). Equilibrium or nonequilibrium: is that the question? *Bulletin of the Ecological Society of America*, *68*(4), 500–502.

Berryman, A. A. (1996). What causes population cycles of forest Lepidoptera? *Trends in Ecology & Evolution*, *11*(1), 28–32.

Berryman, A. A. (2002). Population cycles: causes and analysis. In A. A. Berryman (Ed.), *Population Cycles: The Case for Trophic Interactions* (pp. 3–28). Oxford Univ. Press.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, *144*(3), 353–61.

Bonduriansky, R., Crean, A. J., & Day, T. (2012). The implications of nongenetic inheritance for evolution in changing environments. *Evolutionary Applications*, *5*(2), 192–201.

Bonduriansky, R., & Head, M. (2007). Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *Journal of Evolutionary Biology*, *20*(6), 2379–88.

Boots, M., & Begon, M. (1994). Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth, *Plodia interpunctella*. *Ecological Entomology*, *19*, 319–326.

Boots, M., & Roberts, K. E. (2012). Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proceedings. Biological Sciences / The Royal Society*, *279*(1744), 4009–14.

Burden, J. P., Griffiths, C. M., Cory, J. S., Smith, P., & Sait, S. M. (2002). Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Molecular Ecology*, *11*(3), 547–55.

Burden, J. P., Nixon, C. P., Hodgkinson, A. E., Possee, R. D., Sait, S. M., King, L. A., & Hails, R. S. (2003). Covert infections as a mechanism for long-term persistence of baculoviruses. *Ecology Letters*, *6*(6), 524–531.

Burden, J. P., Possee, R. D., Sait, S. M., King, L. A, & Hails, R. S. (2006). Phenotypic and genotypic characterisation of persistent baculovirus infections in populations of the cabbage moth (*Mamestra brassicae*) within the British Isles. *Archives of Virology*, *151*(4), 635–49.

Burnside, R., Schultz, M., Lisuzzo, N., & Kruse, J. (2013). Assessing mortality and regeneration of Larch (*Larix laricina*) after a 1999-2004 landscape level outbreak of the larch sawfly (*Pristophora erichsonii*) in Alaska (Project WC-EM-08-03). In K. M. Potter & B. L. Conkling (Eds.), *Forest Health Monitoring: National Status, Trends, and Analysis* (pp. 143–150). United States Department of Agriculture - Forest Service.

Carisey, N., & Bauce, E. (2002). Does nutrition-related stress carry over to spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) progeny? *Bulletin of Entomological Research*, *92*(2), 101–8.

Clem, R. J., & Passarelli, a L. (2013). Baculoviruses: sophisticated pathogens of insects. *PLoS Pathogens*, *9*(11), e1003729.

Cook, S. P., Webb, R. E., Podgwaite, J. D., & Reardon, R. C. (2003). Increased mortality of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) exposed to gypsy moth nuclear polyhedrosis virus in combination with the phenolic gycoside salicin. *Journal of Economic Entomology*, *96*(6), 1662–1667.

Cooper, D., Cory, J. S., & Myers, J. H. (2003). Hierarchical spatial structure of genetically variable nucleopolyhedroviruses infecting cyclic populations of western tent caterpillars. *Molecular Ecology*, *12*(4), 881–90. Cooper, D., Cory, J. S., Theilmann, D. A., & Myers, J. H. (2003). Nucleopolyhedroviruses of forest and western tent caterpillars : cross-infectivity and evidence for activation of latent virus in high-density field populations. *Ecological Entomology*, *28*, 41–50.

Corkum, L. D., Ciborowski, J. J. H., & Poulin, R. G. (1997). Effects of emergence date and maternal size on egg development and sizes of eggs and first-instar nymphs of a semelparous aquatic insect. *Oecologia*, *111*, 69–75.

Cory, J. S., Hails, R. S., & Sait, S. M. (1997). *Baculovirus ecology. In: The Baculoviruses (ed. Miller LK)* (pp. 301–339). New York: Plenum Press.

Cory, J. S., & Hoover, K. (2006). Plant-mediated effects in insect-pathogen interactions. *Trends in Ecology & Evolution*, *21*(5), 278–86.

Cory, J. S., & Myers, J. H. (2003). The Ecology and Evolution of Insect Baculoviruses. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 239–272.

Cory, J. S., & Myers, J. H. (2009). Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. *The Journal of Animal Ecology*, *78*(3), 646–55.

Crean, A. J., Dwyer, J. M., & Marshall, D. J. (2013). Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology*, *94*(11), 2575–2582.

De Maagd, R. a, Bravo, A., Berry, C., Crickmore, N., & Schnepf, H. E. (2003). Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. *Annual Review of Genetics*, *37*, 409–33.

Diss, A. L., Kunkel, J. G., Montgomery, M. E., & Leonard, D. E. (1996). Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. *Oecologia*, *106*(4), 470–477.

Donegan, K., & Lighthart, B. (1989). Effect of several stress factors on the susceptibility of the predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen Beauveria bassiana. *Journal of Invertebrate Pathology*, *54*(1), 79–84.

Duffey, S. S., Hoover, K., Bonning, B., & Hammock, B. D. (1995). The impact of host plant on the efficacy of baculoviruses. *Reviews in Pesticide Toxicology*, *3*, 137–275.

Dwyer, G., Dushoff, J., Elkinton, J., & Levin, S. (2000). Pathogen-driven outbreaks in forest defoliators revisited: building models from experimental data. *The American Naturalist*, *156*(2), 105–120.

Dwyer, G., & Elkinton, J. S. (1993). Using simple models to predict virus epizootics in gypsy moth populations. *Journal of Animal Ecology*, *62*(1), 1–11.

Elderd, B. D., Rehill, B. J., Haynes, K. J., & Dwyer, G. (2013). Induced plant defenses, host-pathogen interactions, and forest insect outbreaks. Proceedings of the National Academy of Sciences of the United States of America, 110(37), 14978–14983.

Elliot, N., Kieckhefer, R., & Kauffman, W. (1996). Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia*, *105*, 537–544.

Elton, B. Y. C., & Nicholson, M. (1942). The ten-year cycle in numbers of the lynx in Canada. *Journal of Animal Ecology*, *11*(2), 215–244.

Falconer, D. S. (1965). Maternal effects and selection response. *Genetics Today*, *3*, 763–774.

Ferro, D. N. (1985). Pest status and control strategies of the Colorado potato beetle. *Research Bulletin - Massachusetts Agricultural Experiment Station (USA)*.

Fitzgerald, T. D. (1995). *The Tent Caterpillars*. New York: Cornell University Press.

Fox, C. W. (1997). Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. *Canadian Journal of Zoology*, *75*, 1465–1473.

Fox, C. W., & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, *45*, 341–69.

Fox, C. W., & Mousseau, T. A. (1998). Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. In *Maternal Effects as Adaptations* (p. 159).

Freitak, D., Heckel, D. G., & Vogel, H. (2009a). Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (*Trichoplusia ni*). *Frontiers in Zoology*, *6*, 7.

Freitak, D., Heckel, D. G., & Vogel, H. (2009b). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings. Biological Sciences / The Royal Society*, *276*(1667), 2617–24.

Freitak, D., Wheat, C. W., Heckel, D. G., & Vogel, H. (2007). Immune system responses and fitness costs associated with consumption of bacteria in larvae of *Trichoplusia ni*. *BMC Biology*, *5*, 56.

Frost, P. C., Ebert, D., Larson, J. H., Marcus, M. a, Wagner, N. D., & Zalewski, A. (2010). Transgenerational effects of poor elemental food quality on *Daphnia magna*. *Oecologia*, *162*(4), 865–72.

Fuller, E., Elderd, B. D., & Dwyer, G. (2012). Pathogen persistence in the environment and insect-baculovirus interactions: disease-density thresholds, epidemic burnout and insect outbreaks. *American Naturalist*, *179*(3), 1–30.

Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International journal of molecular sciences* (Vol. 14, pp. 10242–97).

Fuxa, J. R., Sun, J. Z., Weidner, E. H., & LaMotte, L. R. (1999). Stressors and rearing diseases of *Trichoplusia ni*: evidence of vertical transmission of NPV and CPV. *Journal of Invertebrate Pathology*, *74*(2), 149–55.

Gibbs, M., Breuker, C. J., Hesketh, H., Hails, R. S., & Van Dyck, H. (2010). Maternal effects, flight versus fecundity trade-offs, and offspring immune defence in the speckled wood butterfly, *Pararge aegeria*. *BMC Evolutionary Biology*, *10*, 345.

Gillespie, J. P., Kanost, M. R., & Trenczek, T. (1997). Biological mediators of insect immunity. *Annual Review of Entomology*, *42*, 611–643.

Ginzburg, L. R., & Taneyhill, D. E. (1994). Population cycles of forest lepidoptera: a maternal effect hypothesis. *The Journal of Animal Ecology*, *63*(1), 79. doi:10.2307/5585

Gliwicz, Z. M., & Guisande, C. (1992). Family planning in Daphnia : resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia*, *91*(4), 463–467.

Green, B. S. (2008). Maternal effects in fish populations. *Advances in Marine Biology*, *54*, 1–105.

Grimbacher, P., Matsuki, M., Collett, N., Elek, J., & Wardlaw, T. (2011). Are insect herbivores in Eucalyptus globulus/nitens plantations a worsening problem? A multi-region spatio-temporal review of southern Australia. Technical Report No TR216. Cooperative Research Centre for Forestry, Australia..

Grindstaff, J. L. (2008). Maternal antibodies reduce costs of an immune response during development. *The Journal of Experimental Biology*, *211*(Pt 5), 654–60. doi:10.1242/jeb.012344

Grindstaff, J. L., Brodie, E. D., & Ketterson, E. D. (2003). Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings. Biological Sciences / The Royal Society*, *270*(1531), 2309–19. doi:10.1098/rspb.2003.2485

Hafer, N., Ebil, S., Uller, T., & Pike, N. (2011). Transgenerational effects of food availability on age at maturity and reproductive output in an asexual collembolan species. *Biology Letters*, *7*(5), 755–8. doi:10.1098/rsbl.2011.0139

Hajek, A. E., & Leger, R. J. (1994). Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, *39*, 293–322.

Hajek, A. E., McManus, M. L., & Delalibera, I. (2007). A review of introductions of pathogens and nematodes for classical biological control of insects and mites. *Biological Control*, *41*(1), 1–13. doi:10.1016/j.biocontrol.2006.11.003

Hanski, I., Henttonen, H., Korpimaki, E., Oksanen, L., & Turchin, P. (2001). Small-rodent dynamics and predation. *Ecology*, *82*(6), 1505–1520.

Haukioja, E. (2005). Plant defenses and population fluctuations of forest defoliators : mechanism-based scenarios. *Annual Zoology Fennici*, *42*, 313–325.

Haviola, S., Kapari, L., Ossipov, V., Rantala, M. J., Ruuhola, T., & Haukioja, E. (2007). Foliar phenolics are differently associated with *Epirrita autumnata* growth and immunocompetence. *Journal of Chemical Ecology*, *33*(5), 1013–23.

Hoffmann, J. a. (2003). The immune response of Drosophila. *Nature*, *426*(6962), 33–8.

Hoover, K., Washburn, J., & Volkman, L. (2000). Midgut-based resistance of *Heliothis virescens* to baculovirus infection mediated by phytochemicals in cotton. *Journal of Insect Physiology*, *46*(6), 999–1007.

Hoyle, R. B., & Ezard, T. H. G. (2012). The benefits of maternal effects in novel and in stable environments. *Journal of the Royal Society, Interface / the Royal Society*, *9*(75), 2403–13.

Hugar, P., Kulkarni, K. A., & Lingappa, S. (1994). Studies on the transmission of nuclear polyhedrosis virus of sorghum armyworm, *Mythimna separata* (Walker) to its progenies. *Karnataka Journal of Agricultural Sciences*, *7*(4), 417–422.

Huitu, O., Jokinen, I., Korpimäki, E., Koskela, E., & Mappes, T. (2007). Phase dependence in winter physiological condition of cyclic voles. *Oikos*, *116*(4), 565–577.

Hunter, M. D. (2002). Maternal effects and the population dynamics of insects on plants. *Agricultural and Forest Entomology*, *4*(1), 1–9.

Hunter, M. D., & Schultz, J. C. (1993). Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia*, *94*, 195–203.

Il'inykh, A. V, & Ul'yanova, E. G. (2005). Latency of baculoviruses. *Biology Bulletin*, *32*(5), 496–502.

Inchausti, P., & Ginzburg, L. R. (2009). Maternal effects mechanism of population cycling: a formidable competitor to the traditional predator-prey view. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1520), 1117–24.

Marshall, D., & Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, *116*(12), 1957–1963.

Jakubowska, A. K., Vogel, H., & Herrero, S. (2013). Increase in gut microbiota after immune suppression in baculovirus-infected larvae. *PLoS Pathogens*, *9*(5), e1003379.

Janssen, G. M., de Jong, G., Joosse, E. N. G., & Scharloo, W. (1988). A negative maternal effect in springtails. *Evolution*, *42*(4), 828–834.

Jiang, H., Vilcinskas, A., & Kanost, M. R. (2010). Chapter 10 Immunity in Lepidopteran insects. In K. Söderhäll (Ed.), *Invertebrate Immunity* (pp. 181–204). Landes Bioscience and Springer+Business Media.

Kapari, L., Haukioja, E., Rantala, M. J., & Ruuhola, T. (2006). Defoliating insect immune defense interacts with induced plant defense during a population outbreak. *Ecology*, *87*(2), 291–6.

Kessler, A., Poveda, K., & Poelman, E. H. (2012). Plant-induced responses and herbivore population dynamics. In P. Barbosa, D. K. Letourneau, & A. A. Agrawal (Eds.), *Insect outbreaks revisited* (pp. 91–112). Chichester: Wiley-Blackwell.

Kinkel, L. L. (1997). Microbial population dynamics on leaves. *Annual Review of Phytopathology*, *35*, 327–347.

Kinkel, L. L., Wilson, M., & Lindow, S. E. (2000). Plant species and plant incubation conditions influence variability in epiphytic bacterial population size, *Microbial Ecology, 39,* 1–11.

Klemola, N., Heisswolf, A., Ammunét, T., & Ruohomäki, K. (2009). Reversed impacts by specialist parasitoids and generalist predators may explain a phase lag in moth cycles : a novel hypothesis and preliminary field tests, *Annual Zoology Fennici, 2450*, 380–393.

Korner, P., & Schmid-Hempel, P. (2004). In vivo dynamics of an immune response in the bumble bee *Bombus terrestris*. *Journal of Invertebrate Pathology*, *87*(1), 59–66.

Kudo, S. I., & Nakahira, T. (2005). Trophic-egg production in a subsocial bug: adaptive plasticity in response to resource conditions. *Oikos*, *111*(3), 459–464.

Kukan, B. (1999). Vertical transmission of nucleopolyhedrovirus in insects. *Journal of Invertebrate Pathology*, *74*(2), 103–11.

Lacey, L, Frutos, R., Kaya, H. ., & Vail, P. (2001). Insect Pathogens as Biological Control Agents: Do They Have a Future? *Biological Control*, *21*(3), 230–248.

Lambin, X., Petty, S. J., & Mackinnon, J. L. (2000). Cyclic dynamics in field vole populations and generalist predation. *Journal of Animal Ecology*, *69*, 106–118.

Lande, R., & Price, T. (1989). Genetic correlations and maternal effect coefficients obtained from offspring-parent regression. *Genetics*, *122*(4), 915–22.

Leather, S. R. (1985). Oviposition preferences in relation to larval growth rates and survival in the pine beauty moth, *Panolis flammea*. *Ecological Entomology*, *10*, 213–217.

Lee, K. P., Cory, J. S., Wilson, K., Raubenheimer, D., & Simpson, S. J. (2006). Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings. Biological Sciences / The Royal Society*, *273*(1588), 823–9.

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, *69*(4), 1875–1882.

Little, T. J., Connor, B. O., Colegrave, N., Watt, K., & Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology*, *13*, 489–492.

Lotka, A. J. (1932). The growth of mixed populations: two species competing for a common food supply. *Journal of the Washington Academy of Sciences*, *22*, 461–469.

Lynch, M. E., Kaplan, I., Dively, G. P., & Denno, R. F. (2006). Host-plant-mediated competition via induced resistance: interactions between pest herbivores on potatoes. *Ecological Applications16*(3), 855–64.

Lyytikäinen-Saarenmaa, P., & Tomppo, E. (2002). Impact of sawfly defoliation on growth of Scots pine *Pinus sylvestris* (Pinaceae) and associated economic losses. *Bulletin of Entomological Research*, *92*(2), 137–40.

Martemyanov, V. V., Dubovskiy, I. M., Belousova, I. a., Pavlushin, S. V., Domrachev, D. V., Rantala, M. J., … Glupov, V. V. (2012). Rapid induced resistance of silver birch affects both innate immunity and performance of gypsy moths: the role of plant chemical defenses. *Arthropod-Plant Interactions*, (1).

May, R. M. (1973). *Stability and Complexity in Model Ecosystems.* Princeton: Princeton University Press.

Mayer, A. M. (2004). Resistance to herbivores and fungal pathogens: Variations on a common theme? A review comparing the effect of secondary metabolites, induced and constitutive, on herbivores and fungal pathogens. *Israel Journal of Plant Sciences*, *52*(4), 279–292.

Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, *16*(4), 165–167.

McManus, M. L., & Csoka, G. (2007). History and impact of gypsy moth in North America and comparison to recent outbreaks in Europe. *Acta Silvatica et Lignaria Hungarica*, *3*, 47–64.

Meyer, K. M., & Leveau, J. H. J. (2012). Microbiology of the phyllosphere: a playground for testing ecological concepts. *Oecologia*, *168*(3), 621–9.

Meyling, N. V., & Eilenberg, J. (2007). Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control*, *43*(2), 145–155.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings. Biological Sciences / The Royal Society*, *272*(1581), 2601–7.

Molinier, J., Ries, G., Zipfel, C., & Hohn, B. (2006). Transgeneration memory of stress in plants. *Nature*, *442*(7106), 1046–1049.

Moret, Y. (2006). “Trans-generational immune priming”: specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proceedings. Biological Sciences / The Royal Society*, *273*(1592), 1399–405.

Moret, Y., & Siva-Jothy, M. T. (2003). Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proceedings. Biological Sciences / The Royal Society*, *270*(1532), 2475–80.

Mousseau, T. A., & Dingle, H. (1991). Maternal effects in insect life histories. *Annual Review of Entomology*, *36*, 511–534.

Mowlds, P., Barron, A., & Kavanagh, K. (2008). Physical stress primes the immune response of *Galleria mellonella* larvae to infection by *Candida albicans*. *Microbes and Infection / Institut Pasteur*, *10*(6), 628–34.

Müller, T., Müller, M., Behrendt, U., & Stadler, B. (2003). Diversity of culturable phyllosphere bacteria on beech and oak: the effects of lepidopterous larvae. *Microbiological Research*, *158*(4), 291–7.

Murray, K. D., & Elkinton, J. S. (1989). Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). *Journal of Invertebrate Pathology*, *53*(3), 324–334.

Myers, J. H. (1988). Can a general hypothesis explain population cycles of forest lepidoptera? Advances in Ecological Research, 18, 179–232.

Myers, J. H. (2000). Population fluctuations of the western tent caterpillar in southwestern British Columbia. Population Ecology, 42(3), 231-241.

Myers, J. H., & Cory, J. S. (2013). Population cycles in forest lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 131025103132004.

Myers, J. H., Cory, J. S., Ericsson, J. D., & Tseng, M. L. (2011). The effect of food limitation on immunity factors and disease resistance in the western tent caterpillar. *Oecologia*, *167*(3), 647–55.

Myers, J. H., & Kuken, B. (1995). Changes in the fecundity of tent caterpillars : a correlated character of disease resistance or sublethal effect of disease ? *Oecologia*, *103*, 475–480.

Nykänen, H., & Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance : a meta-analysis. *Oikos*, *104*, 247–268.

O'Brien, R. D., & Lindow, S. E. (1989). Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. *Phytopathology*, *79*, 619–627.

Pell, J. K., Eilenberg, J., Hajek, A. E., & Steinkraus, D. C. (2001). Biology, ecology and pest management potential of Entomophthorales. In *Fungi as biocontrol agents: progress, problems and potential* (pp. 71–153). Wallingford: CAB International.

Piene, H., Ostaff, D. P., & Eveleigh, E. S. (2001). Growth loss and recovery following defoliation by the balsam fir sawfly in young, spaced balsam fir stands. *The Canadian Entomologist*, *133*(5), 675–686.

Plaistow, S. J., Lapsley, C. T., & Benton, T. G. (2006). Context-dependent intergenerational effects : the interaction between past and present environments and its effect on population dynamics. *The American Naturalist*, *167*(2), 206–215.

Plymale, R., Grove, M. J., Cox-Foster, D., Ostiguy, N., & Hoover, K. (2008). Plant-mediated alteration of the peritrophic matrix and baculovirus infection in lepidopteran larvae. *Journal of Insect Physiology*, *54*(4), 737–49.

Räsänen, K., & Kruuk, L. E. B. (2007). Maternal effects and evolution at ecological time-scales. *Functional Ecology*, *21*(3), 408–421.

Reynolds, J. J. H., White, A., Sherratt, J. a, & Boots, M. (2011). The population dynamical consequences of density-dependent prophylaxis. *Journal of Theoretical Biology*, *288*, 1–8.

Richards, A., Cory, J., Speight, M., & Williams, T. (1999). Foraging in a pathogen reservoir can lead to local host population extinction: a case study of a Lepidoptera-virus interaction. *Oecologia*, *118*(1), 29–38.

Roberts, D. W. (1973). Means for insect regulation: fungi. *Annals of the New York Academy of Sciences*, *217*(1), 76–84.

Roberts, D. W., & St Leger, R. J. (2004). *Metarhizium spp.*, cosmopolitan insect-pathogenic fungi: mycological aspects. *Advances in Applied Microbiology*, *54*, 1–70.

Romeis, J., Meissle, M., & Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*, *24*(1), 63–71.

Rossiter, M. (1996). Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics*, *27*(1), 451–476.

Rossiter, M. C. (1991). Environmentally-based maternal effects: a hidden force in insect population dynamics? *Oecologia*, *87*(2), 288–294.

Rossiter, M. C. (1994). Maternal effects hypothesis of herbivore outbreak: a framework for the inclusion of population-quality variables as central features of herbivore population-dynamics models. *Bioscience*, *44*(11), 752–763.

Rossiter, M. C., Schultz, J. C., & Baldwin, I. T. (1988). Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology*, *69*(1), 267–277.

Rotem, K., Agrawal, A. A., & Kott, L. (2003). Parental effects in *Pieris rapae* in response to variation in food quality : adaptive plasticity across generations ? *Ecological Entomology*, *28*, 211–218.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. *The Journal of Animal Ecology*, *79*(2), 403–13.

Roth, O., Sadd, B. M., Schmid-Hempel, P., & Kurtz, J. (2009). Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. *Proceedings. Biological Sciences / The Royal Society*, *276*(1654), 145–51.

Rothman, L. D. (1997). Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. *Ecology*, *78*(5), 1481–1493.

Rowley, A. F., & Powell, A. (2007). Invertebrate immune systems specific, quasi-specific, or nonspecific? *Journal of Immunology (Baltimore, Md. : 1950)*, *179*(11), 7209–14.

Roy, B. A., & Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, *54*(1), 51–63.

Saastamoinen, M., Hirai, N., & van Nouhuys, S. (2013). Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. *Oecologia*, *171*(1), 93–104.

Sadd, B. M., & Schmid-Hempel, P. (2006). Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current Biology*, *16*(12), 1206–10.

Sadd, B. M., & Schmid-Hempel, P. (2007). Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Current Biology*, *17*(24), R1046–7.

Sadd, B. M., & Schmid-Hempel, P. (2009). A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumble-bees. *Biology Letters*, *5*(6), 798–801.

Sarfraz, R. M., Cory, J. S., & Myers, J. H. (2013). Life-history consequences and disease resistance of western tent caterpillars in response to localised, herbivore-induced changes in alder leaf quality. *Ecological Entomology*, *38*(1), 61–67.

Schaller, A. (Ed.). (2008). *Induced Plant Resistance to Herbivory*. Springer.

Shah, P. A., & Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, *61*(5-6), 413–423.

Shikano, I., Ericsson, J. D., Cory, J. S., & Myers, J. H. (2010). Indirect plant-mediated effects on insect immunity and disease resistance in a tritrophic system. *Basic and Applied Ecology*, *11*(1), 15–22.

Siva-Jothy, M. T. (2000). A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings. Biological Sciences / The Royal Society*, *267*(1461), 2523–7.

Siva-Jothy, M. T., & Thompson, J. J. W. (2002). Short-term nutrient deprivation affects immune function. *Physiological Entomology*, *27*, 206–212.

Sparks, W. O., Bartholomay, L., & Bonning, B. C. (2008). Insect immunity to viruses. In *Insect Immunology* (pp. 209–242).

Stadler, B., & Müller, T. (2000). Effects of aphids and moth caterpillars on epiphytic microorganisms in canopies of forest trees. *Canadian Journal for Resources*, *30*, 631–638.

Stehr, F. W., & Cook, E. F. (1968). *A revision of the genus Malacosoma Hubner in North America (Lepidoptera: Lasiocampidae): Systematics, biology, immatures, and parasites*.

Steinhaus, E. A. (1956). Microbial control: the emergence of an idea. *Hilgardia*, *26*, 107–160.

Tate, A. T., & Rudolf, V. H. W. (2012). Impact of life stage specific immune priming on invertebrate disease dynamics. *Oikos*, *121*(7), 1083–1092.

Thompson, C. G., Scott, D. W., & Wickman, B. E. (1981). Long-term persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), in forest soil. *Environmental Entomology*, *10*(2), 254–255.

Tidbury, H. J., Pedersen, A. B., & Boots, M. (2011). Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings. Biological Sciences / The Royal Society*, *278*(1707), 871–6.

Tinsley, T. W. (1979). The potential of insect pathogenic viruses as pesticidal agents. *Annual Review of Entomology*, *24*(1), 63–87.

Triggs, A. M., & Knell, R. J. (2012). Parental diet has strong transgenerational effects on offspring immunity. *Functional Ecology*, *26*(6), 1409–1417.

Trudeau, D., Washburn, J. O., & Volkman, L. E. (2001). Central Role of Hemocytes in *Autographa californica* M Nucleopolyhedrovirus Pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. *Journal of Virology*, *75*(2), 996–1003.

Uller, T., Nakagawa, S., & English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *Journal of Evolutionary Biology*, *26*(10), 2161–70.

Underwood, N. (1999). The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist*, *153*(3), 282–294.

Underwood, N. (2010). Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. *Oikos*, *119*(12), 1993–1999.

Valtonen, T. M., Kangassalo, K., Pölkki, M., & Rantala, M. J. (2012). Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. *PloS One*, *7*(2), e31611.

Van Frankenhuyzen, K., Ebling, P., Thurston, G., Lucarotti, C., Royama, T., Guscott, R., … Silver, J. (2002). Incidence and impact of *Entomophaga aulicae* (Zygomycetes: Entomophthorales) and a nucleopolyhedrovirus in an outbreak of the whitemarked tussock moth (Lepidoptera:Lymantriidae). *The Canadian Entomologist*, *134*, 825–845.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. a., Keller, S., … Roy, H. E. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, *2*(4), 149–159.

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., … Lemaitre, B. (2005). *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(32), 11414–9.

Volterra, V. (1926). Variations and fluctuations of the numbers of individuals in animal species living together. *Journal Du Conseil Permanent International Pour l'Exploration de La Mer*, *3*, 3–51.

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, *10*(12), 828–840.

Washburn, J. O., Kirkpatrick, B. A., & Volkman, L. E. (1995). Comparative pathogenesis of *Autographa californica* M nuclear polyhedrosis virus in larvae of *Trichoplusia ni* and *Heliothis virescens*. Virology, 209, 561–568.

Washburn, J. O., Kirkpatrick, B. A., & Volkman, L. E. (1996). Insect protection against viruses. *Nature*, *383*, 767.

Wellington, W. G. (1960). Qualitative changes in natural populations during changes in abundance. *Canadian Journal of Zoology*, *38*, 289–314.

Wellington, W. G. (1965). Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). *The Canadian Entomologist*, *97*(1), 1–14.

Williams, H. L., Monge-Monge, K. S., Otvos, I. S., Reardon, R., & Ragenovich, I. (2011). Genotypic variation among Douglas-fir tussock moth nucleopolyhedrovirus (OpNPV) isolates in the western United States. *Journal of Invertebrate Pathology*, *108*(1), 13–21.

Yang, S., Ruuhola, T., & Rantala, M. J. (2007). Impact of starvation on immune defense and other life- history traits of an outbreaking geometrid, *Epirrita autumnata* : a possible causal trigger for the crash phase of population cycle. *Annual Zoology Fennici*, *44*, 89–96.

Zanchi, C., Troussard, J.-P., Moreau, J., & Moret, Y. (2012). Relationship between maternal transfer of immunity and mother fecundity in an insect. *Proceedings. Biological Sciences / The Royal Society*, *279*(1741), 3223–30.

Chapter 2. Transgenerational effects on disease resistance in the western tent caterpillar

Introduction

An individual's phenotype is determined by interactions between the expression of genes and the influence of environmental factors. While progress in molecular techniques and genomics have greatly advanced the genetic side of this relationship, determining the influences of the environment on phenotype remains challenging. There are a range of environmental factors that can influence individual phenotype, such as food quality and quantity, population density, and temperature (see reviews in DeWitt & Scheiner, 2004; Miner et al., 2005). In addition to the direct influence of the environment on the phenotype of an individual, the conditions experienced by parents can shape offspring phenotypes through changes in egg provisioning (Diss et al., 1996; Benton et al., 2005; Gibbs et al., 2010) or by modifying offspring gene expression (Poulin & Thomas, 2008; Freitak 2009a). Such effects, termed "transgenerational effects", can affect a wide range of invertebrate fitness-related traits (Rossiter, 1991a; Mitchell and Read, 2005; Freitak et al., 2009b; Sadd & Schmid-Hempel, 2009; Frost et al., 2010; Roth et al., 2010; Zanchi et al., 2012) and population dynamics (Mousseau and Fox 1998; Benton et al., 2001; Plaistow et al., 2006). This passage of environmental experience across generations can introduce a lag into a system and result in delayed density-dependent responses (Benton et al., 2001; Triggs & Knell, 2012b). Transgenerational effects can therefore be thought of as feedbacks to population density.

As population density is often positively associated with parasite prevalence and disease transmission (Anderson & May, 1981; McCallum et al., 2001; Lafferty & Holt, 2003; Myers & Cory, 2013), lag introduced through transgenerational effects may be

important for determining the responses of organisms to parasites and pathogens. Research on the invertebrate host-pathogen relationship has principally been focussed on factors that affect the susceptibility of the current generation, though there is increasing evidence that invertebrate disease resistance can be influenced by transgenerational effects related to diet, stress or previous pathogen exposure (Mitchell and Read, 2005; Sadd & Schmid-Hempel, 2009; Frost et al., 2010; Gibbs et al., 2010; Tidbury et al., 2011). While the mechanisms behind invertebrate transgenerational disease resistance are not well understood, it is likely that such phenomena are related to the invertebrate innate immune system.

Although once thought of as simple and hardwired, the innate immune system of invertebrates continues to reveal surprising levels of complexity (Medzhitov & Janeway, 1997; Loker et al., 2004; Little et al., 2005; Vilmos & Kurucz, 1997; Ferrandon et al. 2007; Beckage, 2011). Recent studies indicate that invertebrate immune systems can display adaptive memory-like responses termed 'immune priming' (Moret & Siva-Jothy 2003; Korner & Schmid-Hempel, 2004; Mowlds et al., 2008). In immune priming, exposure to an immune insult or elicitor is believed to upregulate the insect immune system, which may serve to increase resistance to future pathogen challenges. Immune priming can lead to trade-offs between life history and immunocompetence, and could potentially result in increased protection against pathogens (review in Rowely & Powell, 2007; Moret and Siva-Jothy, 2003; Korner & Schmid-Hempel, 2004; Freitak et al. 2007; Mowlds et al., 2008; Sadd & Schmid-Hempel, 2006; Roth et al., 2009b; but see Vorberger et al., 2008; Linder & Promislow, 2009). Immune priming in parents can even act transgenerationally, altering offspring immunocompetence and disease resistance (Little et al., 2003; Moret, 2006; Sadd & Schmid-Hempel, 2009; Freitak et al. 2009a,b).

Immune activation carries substantial costs that can affect host fitness. High resource investment is required for the production of differentiated haemocytes, proteins and enzymes involved in immune pathways, and the antimicrobial peptides/proteins involved in invertebrate immunity (Gillespie et al. 1997; Rowley & Powell, 2007; Jiang et al., 2010). In insects, immune-related resource costs from activation or maintenance are often manifested as energetic costs or as trade-offs with life history metrics (Schmid-Hempel, 2003; Freitak et al., 2003; McKean et al., 2008). Environmental factors such as

poor resource conditions or nutritional stress can therefore influence the insect immune response by limiting resource availability for both immune function and growth and reproduction (Feder et al., 1997; Moret & Schmid-Hempel, 2000; Siva-Jothy & Thompson, 2002; Povey et al., 2009; Myers et al., 2011, Triggs & Knell 2012a,b). Insect immunocompetence can be prioritized over other fitness parameters (Tyler et al., 2006), though such a relationship may be determined by trade-offs between specific immune responses (Rantala & Roff, 2005) or resource availablity (Schmid-Hempel, 2003; Janmaat & Myers, 2005).

Differential immune investment/function may be important for determining the nature and outcome of insect host-pathogen interactions. One important entomopathogen group that may be influenced by the level of immunocompetence is the baculoviruses. Baculovirus epizootics are associated with population declines across a range of Lepidoptera, causing high levels of mortality (Dwyer & Elkinton, 1993; Myers, 2000; van Frankenhuyzen et al., 2002; McManus & Csóka, 2007; Cory & Myers, 2009; Myers et al., 2011; Williams et al., 2011; Elderd et al., 2013; Alalouni et al, 2013). Ecological immunologists measure a number of immune-related metrics, such as haemocyte density, encapsulation response and phenoloxidase activity. Whether any of these immune measures plays a significant role in resistance to baculoviruses is still unclear (Jiang et al., 2010, Saejeng et al., 2010). However, a limited amount of research suggests that haemocytes (involved in the melanisation/encapsulation process) play a role in fighting the initial spread of infection (Trudeau et al., 2001; Washburn et al., 2006; Sparks et al., 2008). The use of molecular tools has demonstrated changes in host gene expression following infection, which could affect the level of host immune responses (Berretta et al., 2013; Jakubowska et al., 2013).

As the invertebrate immune system is resource-dependent, I set out to explore the effects of three resource-related factors that are likely to change with increasing insect density: 1) reduced food availability resulting from defoliation, 2) induced plant responses to feeding damage, and 3) defoliation-related changes in populations of leaf surface microbiota. The influence of resource shortage with rising population density has been shown in a number of invertebrate studies (Beckerman et al., 2002; Mitchell & Read, 2005; Ben-Ami et al., 2010; Boots & Roberts, 2012), including outbreaks of forest

Lepidoptera (Diss et al., 1996; Carisey & Bauce, 2002; Abbot & Dwyer, 2007; Myers & Cory, 2013), Hymenoptera (specifically sawflies) (Piene et al., 2001; Lyytikäinen-Saarenmaa & Tomppo, 2002; Burnside et al., 2013), and Coleoptera (Ferro, 1985; Elliot et al., 1996; Grimbacher et al., 2011). Food limitation generally has negative consequences on both development and immunity in invertebrates within a generation (Donegan & Lighthart, 1989; Siva-Jothy & Thompson 2002; Boggs & Freeman, 2005, Myers et al., 2011; but see Yang et al., 2007). Transgenerational effects of food limitation are more equivocal and depend upon the type of food limitation (i.e. periodic or extended food deprivation, nutrient dilution, or restricted levels of limiting nutrients) (Gliwicz & Guisande, 1992; Mitchell & Read, 2005; Frost et al., 2010; Saastamoinen et al., 2013).

In addition to a plant's constitutive defenses (Wittstock & Gershenzon, 2002), plant defenses can be induced by herbivore damage (Fürstenberg-Hägg et al., 2013; Elderd et al., 2013). Such induced respsonses can be direct or indirect. Direct induced plant defenses can lead to declines in nutritional quality or increases in secondary chemical content of foliage (see reviews in Mayer, 2004; Cory & Hoover, 2006; Schaller, 2008; Fürstenberg-Hägg et al., 2013) and indirect defenses can include the recruitment of natural enemies through plant volatiles (Kessler & Baldwin, 2001). Induced responses can be rapid (i.e. same season) or delayed (previous damage affecting subsequent seasons) (Haukioja et al., 1985; Kaitaniemi et al., 1999; Parry et al., 2003; Nykanen and Koricheva, 2004; Martemyanov et al., 2012a,b). The level of an induced defense can depend on insect herbivore density (Underwood, 1999, 2010; Lynch et al., 2006), with the severity of a plant's response generally increasing with rising levels of herbivore density. Among Lepidopterans, induced plant defenses have been shown to have negative effects on insect development and fecundity (Rossiter et al., 1988; Nykänen & Koricheva, 2004; Sarfraz et al., 2013) but may also upregulate immune function, although this is equivocal (Kapari et al., 2006; Haviola et al., 2007; Martemyanov et al., 2012; Campo et al., 2012). They may also provide protection from entomopathogens (Hunter & Schultz, 1993; Hoover et al., 2000; Sarfraz et al., 2013; but see Cook et al., 2003).

Little is known regarding the effects of induced plant defenses across generations, although a diet of induced foliage has been shown to positively affect offspring mass and decrease the duration of the early instar prefeeding/dispersal stage in the gypsy moth, *Lymantria dispar* (Rossiter, 1991). There are no studies, to my knowledge, that have investigated the transgenerational role of induced plant defenses on offspring immunity or disease resistance. One of the novel aspects of the current study was to assess the presence and direction of any such effects.

The phyllosphere microbial community is another factor that may change with population density in the WTC. Interactions between insect herbivores and leaf surface microorganisms have been little studied, although it has been suggested that insect herbivory can influence the population dynamics of leaf bacteria (Stadler & Müller, 2000; Müller et al., 2003). Stadler and Müller (2000) found that infestation of deciduous trees with lepidopteran larvae resulted in higher abundance of all microorganisms (bacteria, yeast, fungi) on leaf surfaces, though this effect was greatest for bacteria. In a subsequent study, insect herbivory was shown to increase the diversity of leaf surface bacteria (Müller et al. 2003). As consumption of non-pathogenic bacteria has been shown to affect insect fitness and immune function both intra- and transgenerationally (Freitak et al., 2007, 2009a,b), there may also be a link between leaf microbial populations and insect condition.

Consumption of leaf bacteria might affect transgenerational disease resistance, as some studies have found that bacterial priming can enhance immune responses towards homologous infections (Little et al., 2003; Mallon et al., 2003; Sadd & Schmid-Hempel, 2006, 2009; Roth et al., 2010; but see Moret & Siva-Jothy, 2003; Korner & Schmid-Hempel, 2004; Mowlds et al., 2008). Of the few studies that have investigated transgenerational effects on baculovirus resistance in insects (Tidbury et al., 2011; Boots & Roberts, 2012; Shikano et al., in press), none have examined the influence of non-pathogenic bacteria. In the Indian meal moth *Plodia interpunctella*, Tidbury et al. (2011) found that exposure of parents to low doses of *P. interpuntella* granulovirus (PiGV) led to higher virus resistance in both parents and in offspring. However, the study did not account for the potential of sublethal/vertical virus transmission and thus it is not clear whether transgenerational immune priming occurred. In another *P. interpunctella* study,

Boots and Roberts (2012) found that offspring of parents fed a poor diet (increased cellulose concentration) led to offspring with enhanced resistance to PiGV. Recently Shikano et al. (in press), studying the cabbage looper *Trichoplusia ni*, found that a poor parental diet (increased cellulose content) led to offspring with a higher resistance to *T. ni* single nucleocapsid nucleopolyhedrovirus (TnSNPV).

For insect populations, food limitation, induced plant responses, and amplified levels of non-pathogenic leaf bacteria can be viewed as "stressors" that negatively affect individual fitness (Selye, 1950; Brett, 1958), causing fitness trade-offs (Freitak et al., 2007; Campero et al., 2008; Messina & Slade, 1999; Boggs & Freeman, 2005) that alter reproductive allocation and/or gene regulation, and can influence population dynamics (Coors & De Meester, 2008; Hall et al., 2013; Alalouni et al., 2013). Low levels of stress may be compensated for (Moret & Schmid-Hempel, 2000; Campero et al., 2008), but a combination of multiple stressors may impose costs that overwhelm the individual's ability to compensate, leading to increased disease susceptibility (Marcogliese & Pietrock, 2011). When resources are scarce, the ability of parents to adequately provision or prepare progeny may be compromised by the exacerbation of stressor-related costs and offspring fitness can be reduced (Coors & De Meester, 2008; Lorenz & Koella, 2011).

In this study, I tested the transgenerational effects of multiple, density-related dietary stressors (food limitation, induced foliage, and foliar bacteria) on disease resistance in the western tent caterpillar (WTC), *Malacosoma californicum pluviale*. The WTC is a gregarious, tent-forming, polyphagous insect that undergoes population cycles with a periodicity of approximately 6-11 years in southwestern British Columbia (BC), Canada (Myers, 2000). Populations of WTC in BC feed on a variety of deciduous plants, though red alder (*Alnus rubra*) is their primary host. Long-term monitoring of field populations shows that high-density, apparently healthy populations of WTC are rapidly infected with disease, primarily the baculovirus *M. c. pluviale* nucleopolyhedrovirus (McplNPV), causing the population to crash (Cory & Myers, 2009; Myers & Cory, 2013). The source of this virus is intriguing as there can be little evidence of viral disease for several years prior to this epizootic and little is known about how virus persists in WTC populations during low density, trough periods (Cory & Myers, 2009).

What is clear in the WTC system is that an increase in population density is associated with a general decline in larval survival (estimated from the relationship between egg mass size and tent size in the fourth instar) 2-4 years before population peaks are reached, while fecundity remains relatively high up until a year or two later, after which the population peaks and virus epizootics strike (Wellington 1957; Myers 2000; Cory and Myers 2009). It is likely that decreases in larval condition and survival in WTC popultions is linked to increased susceptibility to background pathogens, which can be significant mortality factors for the WTC (Cory & Myers, 2009; Sarfraz et al., 2013). The lag seen between peak larval survival and peak population density in the WTC suggests that delayed density-dependent processes may be at work, increasing host susceptibility to virus epizootics.

Rather than displacing the importance of the host-pathogen relationship and its relation to population cycles in the WTC, the concept of transgenerational effects can be thought of as a subtle but significant link between dynamic environmental factors and the host's susceptibility to pathogens. It was expected that exposure to single stressors would result in fitness costs in parents and result in trade-offs between egg size, number and quality as well as transgenerational trade-offs between enhanced immune function and other fitness aspects (e.g. growth or weight). Additionally, I suspected that bacterial priming in parents would lead to decreased virus resistance due to differences in the expression of immune activity. I propose that exposure to the combination of these stressors would approximate some of the conditions experienced by individual WTC in periods of increasing and high population density, as there is evidence that all three factors can change with increasing herbivore density. The combination of these stressors was predicted to have severe detrimental effects on parents, with combinations exhibiting synergistic effects, leading to offspring with suboptimal development, survival (lower resistance to background pathogens), immunocompetence, and virus resistance (Fig. 2.1). Through this multiple stressor-induced lag effect, we hoped to find a potential mechanism for increased virus susceptibility that characterises the onset of epizootics in high-density WTC populations.

Below I consider the two aspects of this study sequentially. First I consider the impacts of the three stressors on the life history characteristics of the parental

generation. Then I consider the influence of these stressors on the disease susceptibility of the offspring of the “stressed” parents. Finally I discuss how food stress and resulting immunity responses could influence the population dynamics of western tent caterpillars.

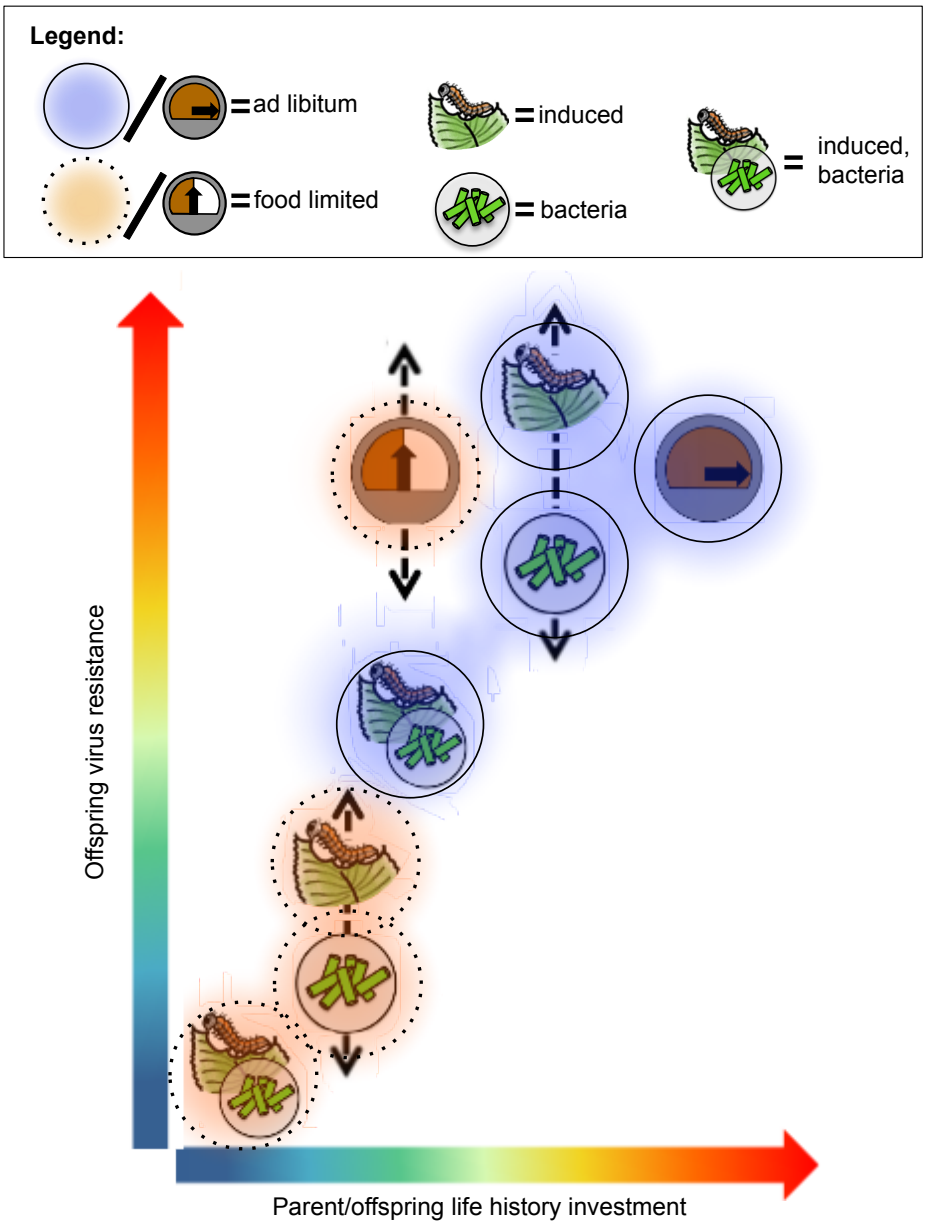


Figure 2.1. General predictions for transgenerational effects of food limitation, induced foliage, and non-pathogenic foliar bacteria on offspring disease resistance and parental/offspring life history investment in *M. c. pluviale*. Warmer colors on the x and y-axis arrows indicate higher levels of each parameter. Single stressors place costs on individuals, lowering investment into growth or reproduction and can modulate transgenerational disease resistance. Double stressors place increasing pressures on parental fitness and might restrict an individual's performance and ability to adequately provision offspring. The triple-stressor treatment at the bottom-left may represent the outcome of simultaneous stressors inflicting negative synergistic effects on parents, leading to offspring with overall weak condition and decreased resistance to virus.

2.A. Parental generation

2.A.1. Parental generation methods

1) Overview of experimental design

To investigate the transgenerational effect of dietary factors on disease resistance in the WTC, I designed a two-year study outlined in Figure 2.2. Parents were reared in a fully factorial (2x2x2) experiment, incorporating the factors of food quantity, plant induction, and leaf microbiota. Life history measurements were recorded for parents, and moths were mated. Egg size and quality, as well as number of eggs per egg mass were measured. Egg masses produced by the parental generation were overwintered and offspring were assayed for susceptibility to McplNPV virus, evaluated for immunocompetence, and monitored for growth and development.

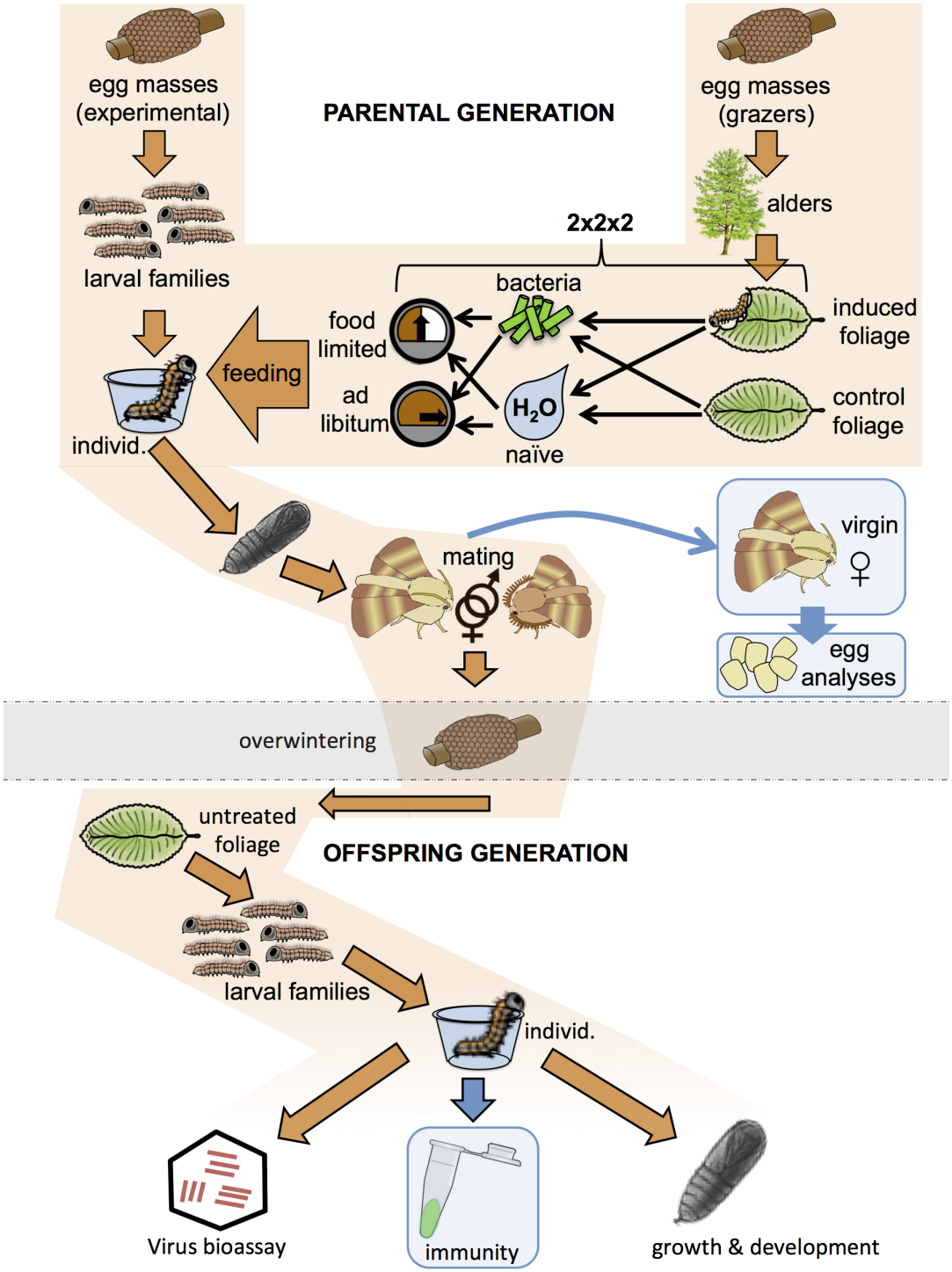


Figure 2.1. Experimental design for study of transgenerational effects on disease resistance in *M. c. pluviale*. Orange represents the flow of the main study; blue symbolizes procedures performed only on ad libitum treatments.

2) Sources of insects

Western tent caterpillar egg masses used for the experiments originated from a field collection made from an apple orchard on Saturna Island, BC in February 2011 (the egg masses would have been laid in the summer of 2010). To remove or reduce transgenerational effects in these field-collected insects, larvae were reared for one generation in family groups in un-crowded conditions on red alder (*A. rubra*) trees on the University of British Columbia (UBC) campus. Naturally occurring western tent caterpillars are uncommon on the UBC campus and there was no history of defoliation on these trees. Insects were brought into the laboratory in the fourth instar and reared through individually on alder leaves, left to pupate and then mated to produce egg masses (Kharouba, 2013). The egg masses were overwintered outdoors in a protected area. Approximately 300 egg masses (each containing around 100-250 eggs) comprised the stock from which experimental larvae were selected. An additional 261 egg masses were collected from Saturna Island on March 31 and April 1, 2012, from red alder trees situated along a roadside. Larvae from these egg masses were deployed to feed on leaves on branches to create induced foliage (see following section for description).

Eggs destined for laboratory experiments were moved from their overwintering site in early April and placed in an incubator at 10/6°C on a 12/12 hr cycle. In mid-April the temperature was gradually increased to allow the larvae to hatch. As early instars only survive when reared in groups and survival is often poor under laboratory conditions, the neonate larvae were placed outside during early development. Families were mixed and groups of 50-150 first instar larvae were transferred to 44 young red alder trees at the Aquatic Research Facility (ARF) on the UBC campus on April 18, 2012. These trees were 2-4 m tall and had no history of tent caterpillar attack.

By May 19, 2012, experimental larvae at the ARF site had reached the mid-late 3rd instar and were collected and kept in groups of 25-50 at 10/8°C in 473 ml waxed paper cups (Solo Cup Company, Highland Park, IL, USA) in a Conviron A-1000 environment chamber (Conviron, Ltd., Winnipeg, MB, Canada). Larvae were fed red alder leaves surface-sterilized with 0.2% bleach solution, collected from non-experimental trees, *ad libitum*. When the majority of the larvae collected from the ARF

site had reached the late third instar they were brought into the laboratory and reared at room temperature (approx. 22-24°C).

3) Parental diet treatments

Manipulation of food quantity

Fourth instar larvae in *ad libitum* treatments were maintained in 60 ml plastic cups and cleaned and fed daily with fresh red alder leaf pieces, while food-limited larvae were fed every other day (Myers et al. 2011). To prevent desiccation of food-limited larvae, a moist piece of paper towel was placed inside each cup on food-free days. Frass was occasionally removed from cups during the 4th instar and removed daily from cups for the duration of the 5th instar.

Creating induced and non-induced foliage

To generate "induced" leaves, branches of red alder trees were subjected to herbivory by WTC larvae. Although no measurements of chemical or nutritional changes in the foliage were taken, herbivore damage is known to influence foliage quality at a branch level, affecting larval development (Sarfraz et al. 2013). Foliage collected from WTC damaged branches was referred to as "induced", while leaves harvested from non-infested branches were designated as "control". The term "control" was meant to signify branches that had only constitutive levels of defense chemicals but were free from herbivore attack. Only undamaged leaves were harvested for feeding to experimental larvae.

In early April 2012, 18 red alder trees in Totem Field at UBC were selected for use in the experiment. On 13 of the trees, four branches per tree were flagged and randomly assigned to herbivore damage or control (two of each). In the remaining five trees only two branches were randomly assigned for damaged or control treatments (one of each) due to limited branch availability.

On April 2, 2012, egg masses destined to be the source of larvae used to create feeding damage were washed in a 0.8% bleach solution for 5 mins to remove any

potentially contaminating microorganisms, rinsed in tap water for an additional 5 mins, then allowed to air-dry before storing in a protected location outside. On April 7, 2012, these egg masses were transferred to the selected branches by attaching two egg masses with zip-ties to branches, approximately 30 cm apart and 30 to 60 cm from the tip of the branch, following the methodology used by Kharouba (2013). Branches were checked regularly and larvae were removed or added to branches as necessary to ensure a balance between sufficient damage and enough leaves for the experiment. At the initial leaf collection, feeding larvae had reached the late 3rd to mid-4th instar and had defoliated branches by approximately 40%. Over the course of the experiment, the level of defoliation gradually intensified, due to the increased feeding by later instars. Tent caterpillars are gregarious and remain within their family group on their tents for most of the larval period. When larvae reached the 5th instar, they dispersed to find pupation sites and had to be removed from branches daily.

Foliage for induced and control treatments was collected daily and leaves from the trees and were randomly assigned to individual larvae within treatments so that larvae were not fed continuously with leaves from the same branches or trees. Leaves were washed in a 0.2% bleach solution for 5 minutes, rinsed in tap water, and air-dried.

Application of foliar bacteria

The bacteria chosen for application to leaves were laboratory-grade *Pseudomonas fluorescens*, *P. syringae* and *Pantoea agglomerans*, all common bacterial species on deciduous leaf surfaces (Hirano & Upper, 2000; Müller et al., 2003; Redford & Fierer, 2009). These were obtained from Z. K. Punja at Simon Fraser University (Burnaby, BC, Canada); bacteria cultures were stored at -80°C in a glycerol (Caledon, Georgetown, ON, Canada) solution.

Approximately 11 days from the start of feeding parental larvae on experimental diet treatments (i.e. fully factorial combinations of food quantity, leaf quality, and presence/absence of foliar bacteria), bacterial cultures from frozen glycerol stocks were plated onto nutrient agar and allowed to grow for 3 days at room temperature. After this, several colonies from each plate were collected and used to inoculate 15 ml glass vials each containing 5 ml liquid nutrient broth (NB powder (Fluka Analytical, MO, USA), 4 g/L

DI H_2O; 6 vials per species). Vials were shaken for approximately 18 hr at room temperature to incubate cultures.

One week prior to the start of diet treatments, 10 ml aliquots of all three bacterial species were made to prepare the mixed bacterial suspension for leaf dipping. From each of the previously incubated bacterial cultures 1 ml was taken for OD (optical density) measurements at 600 nm in a Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) using a standard cuvette. Based on serial dilutions of previous bacterial suspensions, OD readings for estimated colony forming units (CFU) per ml were predicted to be approximately 1 x 10^9 (*Pseudomonas fluorescens*, *Pantoea agglomerans*) and 1 x 10^8 (*Pseudomonas syringae*) CFU/ml. In order to check the viability of frozen aliquots and accuracy of OD readings, serial dilutions of samples were plated (4 reps x 3 dilutions x 3 bacterial species) to determine colony survival (unpublished data).

The remainder of the incubated culture vials were used to prepare bacterial suspensions made up of equal portions of the three bacterial species (20 ml each of 1 x 10^9 CFU/ml – *P. syringae* cultures were concentrated by a factor of 10 to achieve this concentration) based on the OD estimates. From this mix, 60 aliquots were made. For each aliquot, 1 ml of the mixture was pipetted into a sterile 15 ml polypropylene conical tube (Corning Inc., Corning, NY, USA) and 7.5 ml of sterile water and 1.5 ml glycerol were added. The aliquots were frozen at -20°C.

The chosen concentration for leaf dipping was 1 x 10^5 CFU/ml. This was based on: 1) selecting a concentration of common phylloplane bacteria found on deciduous leaf surfaces in the field, and 2) inducing an effect in larvae without causing mortality. In summer months Stadler and Müller (2000) found that concentrations of common phylloplane bacteria on oak and beech trees ranged anywhere from 1 x 10^4 CFU/g to 1 x 10^7 CFU/g (CFU/g measurements are approximately equal to CFU/ml). To address the second concern, there is little empirical evidence on the effect of these bacteria on insects. However, Sadd and Schmid-Hempel (2006) found that all naïve bumblebees cleared injections of 5 x 10^4 CFU/ml of live *P. fluorescens*, while a higher dose at 2.5 x

10^6 CFU/ml resulted in approximately 10% mortality. Based on these two studies, it was decided that concentration of 1 x 10^5 CFU/ml would adequately address both concerns.

Prior to dipping the leaves, a bacterial aliquot was thawed, vortexed for several seconds and 1 ml portions were then pipetted into a 1.5 ml eppendorf tubes, which were centrifuged at 14,000 rpm for 20 mins to pellet the bacteria. After removing the supernatant, 1 ml water was added to each tube, which was then vortexed for several seconds before pipetting into a beaker. The tubes were then rinsed out with an additional 1 ml of water, which was added to the beaker. Water (199 ml) was subsequently added to the beaker to attain the correct concentration of dipping suspensions.

Leaves were left to dry for approximately 45 – 60 mins after the bleach rinse, and then dipped in either a beaker of water (naïve) or bacterial cocktail. The entire leaf was immersed in liquid for 2-3 seconds using sterile forceps and air-dried.

4) Parental generation experimental timeline

Within 24 hr of moulting into the fourth instar 1600 caterpillars were haphazardly selected from cups containing mixed family groups, weighed, and placed in individual pre-labeled 60 ml plastic soufflé cups (Solo Cup Company, Highland Park, IL, USA). Eight treatment groups (full factorial 2 x 2 x 2) were created, with 200 larvae in each group: 1) induced and bacteria dipped foliage, food limited; 2) induced and naïve (no bacteria added) foliage, food limited; 3) control (non-induced) and bacteria dipped foliage, food limited; 4) control and naïve foliage, food limited; 5) induced and bacteria dipped foliage, ad libitum; 6) induced and naïve foliage, ad libitum; 7) control and bacteria dipped foliage, ad libitum; 8) control and naïve foliage, ad libitum.

The experiment was set up over three consecutive days (May 23-25) as different groups of larvae moulted into the fourth instar and on each day the newly moulted larvae were spread evenly among the eight treatments. All treatments were applied immediately after assigning larvae to individual containers. Once dry, leaves were cut into small pieces ranging from approximately 3 - 16 cm^2, depending on instar and larval food demands.

Insects were checked daily and when they moulted to the 5th instar, larvae were weighed the following morning (food limited: n = 770; ad libitum: n = 781). The larvae were then reared through to pupation. The date of cocoon formation was noted and pupae were sexed and weighed 3 days later (food limited: n = 316; ad libitum: n = 752). After weighing, pupae were kept hydrated by misting with water every other day, starting 6 days after cocoon formation. If larvae failed to form a cocoon but still pupated, an estimate was made as to the equivalent date they would have formed a cocoon. Pupae were kept for moth emergence, mating, and oviposition.

5) Measurements of parental life history and background mortality

Basic life history measurements were recorded during the parental generation. Growth rate and development time were measured between moulting to the 4th instar and pupation. Background occurence of McplNPV infection resulted in larvae displaying characteristic NPV symptoms (fragile cuticles, cadavers oozing milky liquid) (Cory & Myers, 2009). In cases where McplNPV induced mortality was not obvious, larval or pupal cadavers were smeared and screened for the presence of viral occlusion bodies (microscopic examination using phase-contrast under 1000x magnification; Nikon H550L compound microscope); observable symptoms of non-viral deaths were recorded, including abnormalities in size, shape, activity, or the presence of fungal growth.

6) Mating and egg production

Moths emerged between June 19 and July 3. After emergence, females were transferred individually to a 473 ml waxed paper cup with a freshly cut twig of red alder (approximate diameter: 1.5 – 2.0 cm; approximate length 10 cm). When males emerged, they were transferred to a mating cup with a female or stored at 12°C in a 60 ml plastic cup until needed. Moths were mated within treatment. Insects usually mated within the first 24 to 48 hrs. Successful matings were recorded and egg masses were labeled with the mating pair's ID. Unsuccessful pairs were given a fresh alder twig and if necessary the male was replaced.

Egg masses (food limited: n = 18; ad libitum: n = 186) were stored at room temperature and misted with water daily until early July. They were then placed inside

mesh nylon bags (1 bag per treatment) and hung inside a screened box with a solid wood top and bottom located outdoors to undergo winter diapause. The box was rotated periodically and mesh bags containing egg masses were moved occasionally within the box to equalize any sun exposure or thermal variation within the box.

7) Egg analysis and fecundity in gravid females

From each *ad libitum* treatment, approximately 30 gravid females were selected randomly among moths emerging from June 23 – 25 and frozen at -20°C for later egg analyses. There were insufficient insects in the food-limited treatments to do this.

Egg count and volume

Frozen gravid virgin females collected from the ad libitum treatments were dissected and eggs were counted, measured and refrozen for later analyses. A subsample of 20 eggs was randomly chosen from the total mass to be measured (n = 121). Eggs were placed on a glass slide and pictures were taken under a dissecting scope (Nikon SM21500) at 10x using a camera (Nikon Digital Camera DXM1200F). Next, ImageTool (v. 3.0, UTHSCSA, TX, USA) was used to measure each egg's length and width. Egg shape in the WTC approximates a cylinder and from the measurements taken, an estimate of each egg's volume as a cylinder was calculated ($V = \pi r^2 h$). Egg batches were then divided into two 1.5 ml eppendorf tubes and were frozen at -80° C until further analysis.

Egg protein and lipid analyses

The subsample of 20 eggs measured for volume was used to estimate average amounts of protein (n = 96) and lipids (n = 101) contained within eggs. After thawing, the eggs were washed as described by Geister et al. (2008) to remove any possible contamination from the mother's abdominal fluids. Briefly, 250 µl sterile water were added and the eggs were washed for 10 s in an ultrasonic bath (B-5200R-1, Branson Cleaning Co., Shelton, CT, USA). The water was then removed and 500 µl CHCl3:MeOH (1:1) was added to each sample tube. Tubes were ultrasonicated again for 10 s. The eggs were then rinsed twice with 1 ml sterile water using a borosilicate filter

membrane funnel attached to an Erlenmeyer flask with a side arm vacuum. They were then placed into a drying oven for 36 h at 50° C. After drying, eggs were homogenized manually using a plastic pestle in 100 µl CHCl3:MeOH (2:1).

Egg protein analysis was done using the Bradford method (Bradford, 1976). The homogenates were vortexed briefly and 10 µl of each sample was added to a 96 well microplate (Falcon, Abilene, TX, USA). Bovine serum albumin (BSA) standards (Biorad, Hercules, CA, USA) (0, 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2 mg/ml) were pipetted in duplicate on each microplate. Bradford reagent (200 µl) was added to the homogenates and standards and the microplates were incubated for 15 mins at room temperature. Absorbance was read on a spectrometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA) at 595 nm. A standard curve was generated from the standard absorbance values and sample protein levels were estimated in mg/ml. In order to control for absorbance readings due to solvent in samples, 10 µl CHCl3:MeOH (2:1) was pipetted into several wells per microplate and average values obtained from these blanks were subtracted from sample absorbance values. To determine the amount of protein contained per egg (mg), average egg volume (ml) measurements were multiplied by estimated levels of protein (mg/ml).

A modified sulpho-phospho-vanillin method was used to analyze the lipid content of eggs (following Pöykkö and Mänttäri, 2012 and employing elements of Cheng et al., 2011). Ten microliters of the previously prepared egg homogenates and duplicate olive oil standards (0, 1, 2, 4, 9, 17, 35 µl oil/100 µl solvent) were added to wells of a 96 well microplate and kept at 90° C for approximately 10 mins to allow solvent to evaporate. Next, 100 µl sulfuric acid (95%) was added to each well and incubated at 90° C for 20 mins, after which the microplates were immediately placed in ice water for 2 mins. After cooling, background absorbance was read at 530 nm. Then, 50 µl phospho-vanillin reagent (0.2 mg vanillin per ml of 17% phosphoric acid) was added to each well and incubated for 10 mins at room temperature. Samples were then measured alongside standards and blanks at 530 nm in a spectrometer. A standard curve was made based on olive oil standards and background absorbance values were subtracted from standard and sample absorbance values. Lipid content (mg/ml) was extrapolated from

standard curves. To determine the amount of lipid contained per egg (mg), average egg volume (ml) was multiplied by extrapolated levels of lipid (mg/ml).

Egg antibacterial enzyme activities

Tubes containing the remainder of each female's eggs were used to investigate egg antibacterial activity (n = 105) using procedures modified from Moreau et al. (2012) and Ericsson et al. (2009). Antibacterial activity was tested against two separate species of bacteria, the Gram-positive *M. luteus* (lyophilized cells, No. 4698, Sigma-Aldrich, St. Louis, MO, USA) and the Gram-negative *E. coli* (strain K-12; obtained from C. A. Lowenburger, Simon Fraser University). Twenty eggs were selected at random to obtain an average antibacterial activity from each female's clutch. Eggs were placed into 0.5 ml eppendorf tubes and 250 µl H_2O was added. Tubes were then ultrasonicated to gently remove maternal body fluids from egg surfaces for approximately 10 seconds. Eggs were homogenized with a pestle in 10 ul PBS (8.74 g NaCl, 1.78 g $Na_2HPO_42H_2O$, 1000 ml distilled water, pH 6.5) and then briefly centrifuged and vortexed before 2 µl of homogenate was added to the two kinds of antibacterial assay plates.

To make *M. luteus* plates, methodology followed that of Wilson et al. (2002). In brief, 9 cm diameter plates were made with 10 ml of 1% agar with 5 mg/ml freeze-dried *M. luteus* mixed with 0.1 mg/ml streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA), and 67 mM potassium phosphate buffer (pH 6.4).

E. coli plates were prepared from fresh bacterial cultures. *E. coli* were taken from a frozen glycerol stock and grown overnight in LB broth (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) at 37° C with shaking. One ml was added to 100 ml warm LB broth and grown for approximately 1 hr to mid-log phase. Optical density was determined for the mid-log culture (OD_{600} = 0.2) and serial dilutions were plated on LB agar plates (7.5 g agar/L LB broth) to determine the bacterial concentration. From the mid-log culture, 1 ml/per 10 ml plate was added to molten LB agar at approximately 40° C and the mixture was poured into 9 cm petri dishes and allowed to cool.

Both *M. luteus* and *E. coli* plates were stored at 4° C for up to 24 h but were warmed prior to use to approximately 33 and 37° C, respectively. Small holes (2-mm

diameter) were punched in the agar. Two µl of a mixture containing thawed haemolymph and PBS buffer (1:1) were pipetted into wells. For *M. luteus* plates, hen egg white lysozyme (Fluka Analytical, St. Louis, MO, USA) was used as a standard to obtain standard curves, while *E. coli* plate measurements were standardized with ampicillin (obtained from C. A. Lowenburger, Simon Fraser University). At least two sets of standards were made for each batch of plates (*M. luteus* dilution range: 1, 0.75, 0.5, 0.25, 0.125 mg/ml; *E. coli* dilution range: 5, 2.5, 1.25, 0.625, 0.313 mg/ml) and one of the standards (*M. luteus*: 0.125 mg/ml; *E. coli*: 0.313 mg/ml) was added to each sample plate to calibrate diameters of the lytic zones.

M. luteus and *E. coli* plates were incubated for 24 h at 33° C and 37° C, respectively. After incubation, zones of inhibition were measured with Vernier calipers to the nearest 0.1 mm.

8) Parental moth fecundity

Each egg mass (food limited: n = 18; ad libitum: n = 186) produced by mating within the parental generation treatments was counted post-hatch, recording both number hatched and the total number of eggs.

9) Statistical analysis

Fourth instar mass was initially included as a covariate in analyses of pupal mass, development time, and survival but initial mass was removed from final models if non-significant. Growth rate was estimated with the formula for relative growth rate (RGR), represented by Equation 1 (where M_m is final mass, M_o is initial mass, and t is time interval between recording initial and final mass):

Equation 1. Relative growth rate = $\ln(M_m / M_o)/t$

Selection of final model terms was done by fitting all higher-order terms and removing non-significant terms sequentially. Significant interaction terms for general linear models were assessed with Tukey's HSD at $P < 0.05$. Interactions occurring with generalized linear models were explored through the use of contrast tests. Means, ±1

standard error, are given. Unless stated, statistics were performed using JMP (SAS Institute Inc. 2012), otherwise RStudio (0.97.310).

Values expressed in the results section (text, figures, or tables) are generally given as least square (LS) means, though some estimates are derived from GLM models or raw data, depending on the particular analysis.

Life history parameters in the parental generation were strongly affected by food quantity, which dwarfed the effects of plant induction and leaf microbiota, causing overall distributions to be non-normal (bi-modal) and a suitable transformation could not be applied. To evaluate the effect of food quantity on the various life history parameters, non-parametric one-way Wilcoxon tests were employed. The more subtle effects of plant induction and leaf microbiota were explored through separate analyses of full and half food treatments using general linear models. Parental pupal masses were analyzed separately by sex, as WTC are strongly sexually dimorphic with females being larger. Pupal mass of food-limited females was $\log_{10}$ transformed to attain a normal distribution. Parental survival was analyzed on a fully factorial basis (2x2x2) using a generalized linear model with a binomial error structure and a logit-link function and a survival analysis was performed using a Cox Proportional Hazards model.

Egg size and composition was analysed using a factorial analysis including plant induction and leaf microbiota (2x2). Subsamples of individual eggs collected from dissected virgin females for size measurements were nested with female, and female ID was treated as a random effect. Female pupal mass was included as a covariate in analyses of fecundity and egg composition, but was removed from models if non-significant. For egg size, egg count was included as a covariate. Egg protein, lipid, and lysozyme equivalent measurements were $\log_{10}$ transformed.

Fecundity was analyzed first for food quantity (as a single factor) and then separately for ad libitum and food limited treatments for plant induction and leaf microbiota (2x2). For ad libitum treatments, egg counts for dissected virgin females and egg masses left to hatch were combined. Within ad libitum females, total egg count data were reflected, $\log_{10}$ transformed, and re-reflected to attain a normal distribution (see Osborne, 2002).

2.A.2. Parental generation results

1) Abbreviations and terms used in results and figures

For simplicity and to save space, abbreviations are used in the text and figures. First, figures use the following abbreviations: Induced = induced foliage, Control = control foliage, B = bacteria dipped leaves, N = naïve leaves. Second, figures and text use abbreviations for treatment groups based on plant induction and leaf microbiota status. Abbreviations are as follows: IB = induced, bacteria dipped foliage, IN = induced, naïve foliage, CB = control, bacteria dipped foliage, CN = control, naïve foliage.

"Food quantity" refers to whether the larvae were fed daily or every other day: these levels are referred to as "ad libitum" and "food limited" respectively. "Plant induction" or "induction" refer to the use of foliage from branches with or without herbivore damage, and levels are referred to as "induced" and "control". The treatment which involved dipping leaves into bacterial suspensions or water prior to feeding, is termed "leaf microbiota" or "microbiota". Levels within leaf microbiota are referred to as "bacteria" and "naïve."

2) Presentation of results

Food limitation strongly affected survival and life history metrics in the parental generation. Therefore, results for food quantity are reported first, followed by those for plant induction and leaf microbiota analyzed separately for the food limited and ad libitum groupings.

3) Effects of food quantity on parental life history

Overall, food limitation had a huge negative impact on pupal mass and reduced it by approximately 50% in both sexes (Table 2.1). In addition, growth rate declined by approximately 50% and development time increased by around 30% in larvae that were food limited compared to larvae fed ad libitum (Table 2.1). Survival in the parental generation was greatly reduced by food limitation (Table 2.1). Females fed reduced food as larvae laid fewer eggs per egg mass compared to females fed ad libitum as larvae (Table 2.1).

Table 2.1. Effects of food quantity on life history characteristics of the *M. c. pluviale* parental generation. Abbreviations "fd lim" and "ad lib" stand for food limited and ad libitum, respectively; n = sample size. P values in bold represent statistically significant effects at P<0.05.

Parameter	fd lim (± 1 SE)	ad lib (± 1 SE)	n (fd lim)	n (ad lib)	Statistics
♀ Pupal mass*	244.2 mg (6.0)	506.5 mg (2.9)	78	338	χ^2=183.2, **P<0.001**
♀ Rel. growth rate*	0.058 (0.002)	0.118 (0.001)	78	337	χ^2=184.1, **P<0.001**
♀ Dev. time*	28 days (0.3)	20.5 days (0.1)	78	340	χ^2=184.2, **P<0.001**
♂ Pupal mass*	158.5 mg (2.2)	279.0 mg (1.4)	240	420	χ^2=439.8, **P<0.001**
♂ Rel. growth rate*	0.053 (0.001)	0.108 (0.001)	240	418	χ^2=445.7, **P<0.001**
♂ Dev. time*	27 days (0.18)	19 days (0.1)	240	421	χ^2=444.3, **P<0.001**
Surv. to emerg.†	23.6 % (1.5)	92.1 % (1.0)	795	804	χ^2=901.0, **P<0.001**
Fecundity*	81 eggs (7.4)	218 eggs (2.2)	18	186	χ^2=48.9, **P<0.001**

*Statistics, means, & SEs reported from Wilcoxin one-way χ^2 approximation
†Statistics, means, & SEs reported from binomial logit GLM

4) Effects of induction and leaf microbiota on parental life history traits

Parental pupal mass

Separate analyses of the food limited and ad libitum treatments indicated that overall, plant induction had little impact on pupal mass. However, the presence of leaf microbiota was detrimental to parents, but varied with both food quantity and sex.

i) Food-limited treatments

Ingesting phylloplane bacteria had a clear effect on food-limited insects, with both female and male pupae being ~15% smaller (Females, microbiota: $F_{1,74}$ = 12.889, P < 0.001 and Males, microbiota: $F_{1,235}$ = 48.202, P < 0.001) (Fig. 2.3a,b). Plant induction had no impact on the mass of females ($F_{1,74}$ = 1.059, P = 0.307); however, males were slightly *heavier* when fed induced foliage ($F_{1,235}$ = 4.540, P = 0.034) (Fig. 2.3b). There was no interaction between plant induction and leaf microbiota for either sex (Females, induction*microbiota: $F_{1,72}$ = 0.012, P = 0.915; Males, induction*microbiota: $F_{1,234}$ = 0.021, P = 0.885). Initial larval mass was positively correlated with pupal mass in both sexes, though the relationship was only significant for males ($F_{1,235}$ = 21.18, P < 0.001) but not females ($F_{1,73}$ = 3.201, P = 0.078).

ii) Ad libitum treatments

Neither plant induction nor leaf microbiota influenced female pupal mass in the ad libitum treatments (plant induction: $F_{1,334}$ = 0.030, P = 0.862; leaf microbiota: $F_{1,334}$ = 0.669, P = 0.414). There was no interaction between the two factors (induction*microbiota: $F_{1,331}$ = 1.200, P = 0.274), and initial larval mass had no influence ($F_{1,332}$ = 1.980, P = 0.160) (Fig. 2.3c). However, males fed induced foliage plus bacteria weighed less than males fed on induced foliage without bacteria or control foliage plus bacteria, but did not differ from males reared on bacteria-free control foliage (induction*microbiota: $F_{1,408}$ = 7.417, P = 0.007) (Fig. 2.3d). In addition, there was a close to significant trend for males reared on bacteria treated foliage to be smaller than males fed naïve foliage ($F_{1,408}$ = 3.657, P = 0.057) (Figure 2.3d). There was no main effect of plant induction on male pupal mass ($F_{1,408}$ = 2.624, P = 0.106). Initial mass was positively correlated with pupal mass ($F_{1,408}$ = 10.684, P = 0.001) for males.

Parental growth rate and development time

Growth rate and development time were influenced by both plant induction and leaf microbiota, though their impact depended on food quantity. Food-limited treatments experienced a clear negative effect on growth and a prolonged development time when bacteria were applied to induced foliage.

i) Food-limited treatments

Relative growth rate of food-limited larvae was strongly affected by leaf microbiota, but was not influenced by plant induction (Fig. 2.4; Table 2.2). Females grew faster than males (Table 2.2). The interaction between plant induction and sex was close to significance, and suggested that female growth was more negatively affected by induced foliage than male growth (Table 2.2).

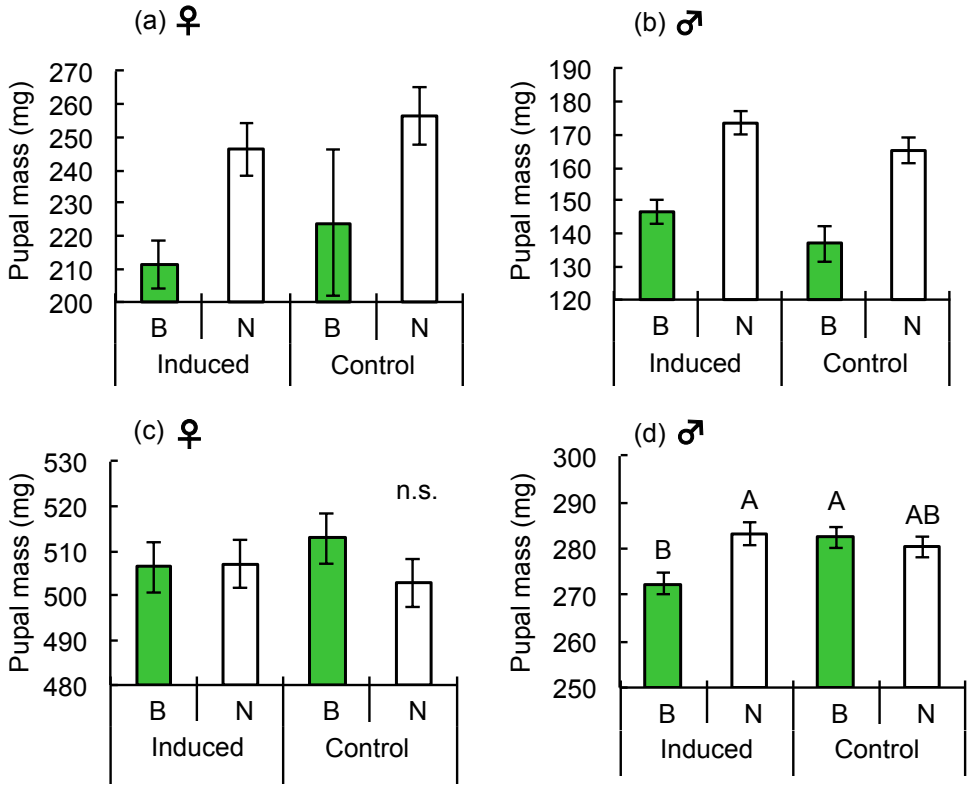


Figure 2.2. Pupal mass of *M. c. pluviale* parents for (a) food-limited females (n = 77), (b) food-limited males (n = 239), (c) ad libitum females (n = 337), and (d) ad libitum males (n = 413). Bars in (b), (c), and (d) show treatment LS means (± 1 SE); values shown in (a) are based on Log_{10} backtransformed values. Uppercase letters above bars represent a statistically significant interaction between treatment means (Tukey's test at $P<0.05$); n.s.= non-significant. Levels with shared letters are not statistically different. "Induced" refers to a diet of foliage from larvae-infested branches, while "Control" denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

Development time mirrored the effects seen for growth rate, with larvae fed bacteria treated foliage taking longer to develop compared to larvae fed naïve foliage (Fig. A1; Table A1). Plant induction had no effect on development time (Fig. A1; Table A1). Females took longer to develop than males, and there was a negative relationship between initial mass and development time (Table A1). There were no interactions between any of model parameters (Table A1).

Table 2.2. Final minimal statistical model for growth rate (4th instar - pupation) for food-limited *M. c. pluviale* parents (n = 316). P values in bold represent statistically significant (P < 0.05) and marginally non-significant effects. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	*F*	P
Induction	1	1.075	0.301
Microbiota	1	67.043	**<0.001**
Sex	1	7.983	**0.005**
Induction*sex	1	3.422	**0.065**
Error	312		

Deleted terms: Microbiota*sex: $F_{1,310}$=0.719, P=0.397; induction*microbiota: $F_{1,309}$=0.016, P=0.901; induction*microbiota*sex: $F_{1,308}$=0.333, P=0.565.

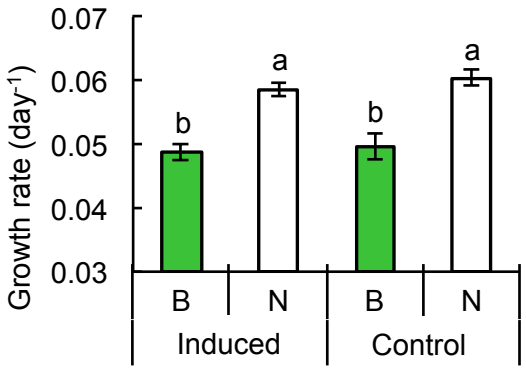


Figure 2.3. Growth rate (4th instar - pupation) for food-limited *M. c. pluviale* parents (n = 316). Estimates are given as LS Means (± 1 SE). "Induced" refers to a diet of foliage from larvae-infested branches, while "Control" denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application). Lowercase letters indicate a significant main effect from the application of foliar bacteria; shared letters indicate no difference between treatments.

ii) Ad libitum treatments

Insects fed ad libitum grew more slowly when they were fed induced foliage compared to control foliage (Table 2.3a). There was no main effect of leaf microbiota; however, there was a strong interaction between plant induction and leaf microbiota,

which indicated that the IB treatment had a lower growth rate than the other three treatments (Fig. 2.5a; Table 2.3a). Females grew faster than males (Table 2.3a).

As expected, development time showed a similar pattern with larvae taking slightly longer to mature when fed induced foliage compared to control foliage (Table 2.3b). Larvae took longer to develop on IB foliage compared IN and CB foliage, but did not differ from insects reared on CN foliage (Fig. 2.5b; Table 2.3b). Females took longer to develop than males, and there was a negative relationship between initial larval mass and development time (Table 2.3b).

Table 2.3. Final minimal statistical model for *M. c. pluviale* pluviale parental generation (a) growth rate and (b) development time (4th instar - pupation) for ad libitum larvae (n = 752). P values in bold represent statistically significant effects. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

a) Growth rate

Source	d.f.	*F*	P
Induction	1	11.191	**0.001**
Microbiota	1	1.924	0.166
Induction*microbiota	1	22.323	**<0.001**
Sex	1	97.495	**<0.001**
Error	747		

Deleted terms: Induction*sex: $F_{1,746}$=0.661, P=0.417; microbiota*sex: $F_{1,745}$=0.238, P=0.626; induction*microbiota*sex: $F_{1,744}$=0.376, P=0.540.

b) Development time

Source	d.f.	*F*	P
Induction	1	4.016	**0.045**
Microbiota	1	2.614	0.106
Induction*microbiota	1	20.843	**<0.001**
Sex	1	309.66	**<0.001**
Initial mass	1	115.61	**<0.001**
Error	746		

Deleted terms: Induction*sex: $F_{1,745}$=2.132, P=0.145; microbiota*sex: $F_{1,744}$=0.630, P=0.428; induction*microbiota*sex: $F_{1,743}$=0.021, P=0.885.

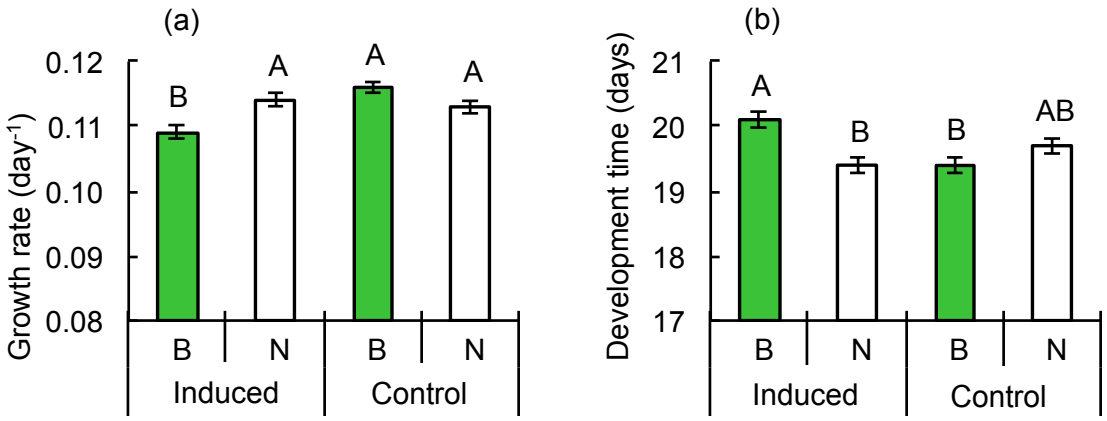


Figure 2.4. (a) Growth rate and (b) development time (4th instar - pupation) for ad libitum larvae of *M. c. pluviale* parental generation (n = 752). Estimates are given as LS Means (± 1 SE). Uppercase letters above bars represent a statistically significant interaction between treatment means (Tukey's test at P<0.05); Levels with shared letters are not statistically different. "Induced" refers to a diet of foliage from larvae-infested branches, while "Control" denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

Parental survival

Parental survival (fully factorial, 2x2x2) was strongly affected by food quantity (food-limited: ~25% survival; ad libitum ~90% survival; Table 2.4]) and declined markedly in all food limited treatments starting at around day 23 of the experiment, when most starved insects were nearing the prepupal stage (Fig. 2.6). There were no overall effects of plant induction or leaf microbiota; however, there was a significant interaction (Table 2.4), which indicated that, regardless of food quantity, CB larvae had the lowest survival compared to all other combinations.

For the food-limited larvae, the principal sources of mortality occurred late in development, especially as insects neared transition to the pupal stage. Sources of mortality included: fungal infection (~42%), pupation failure (~22%), unknown causes (~7%), NPV (~4%), and parasitoids (~2%). Mortality in the ad libitum treatments was low (< 10%) with the primary source of mortality being pupation failure (~5%), although a few insects were killed by parasitoids (~2%), NPV (<1%), and unknown causes (~1%).

Table 2.4. Final statistical model for survival to emergence for *M. c. pluviale* parents, analyzed with a Cox proportional hazards model (n = 670). P values in bold represent statistically significant effects at P<0.05. "Quantity" represents whether larvae were fed every other day (food limited) or daily (ad libitum), "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	df	χ^2	P
Quantity	1	16.089	**<0.001**
Induction	1	2.490	0.115
Microbiota	1	1.794	0.180
Induction*microbiota	1	5.062	**0.025**

Deleted terms: Initial mass: χ^2=1.828, df=1, P=0.176; quantity*microbiota: χ^2=0.315, df=1, P=0.575; quantity*induction: χ^2=0.024, df=1, P=0.878; quantity*induction*microbiota: χ^2=0.553, df=1, P=0.457.

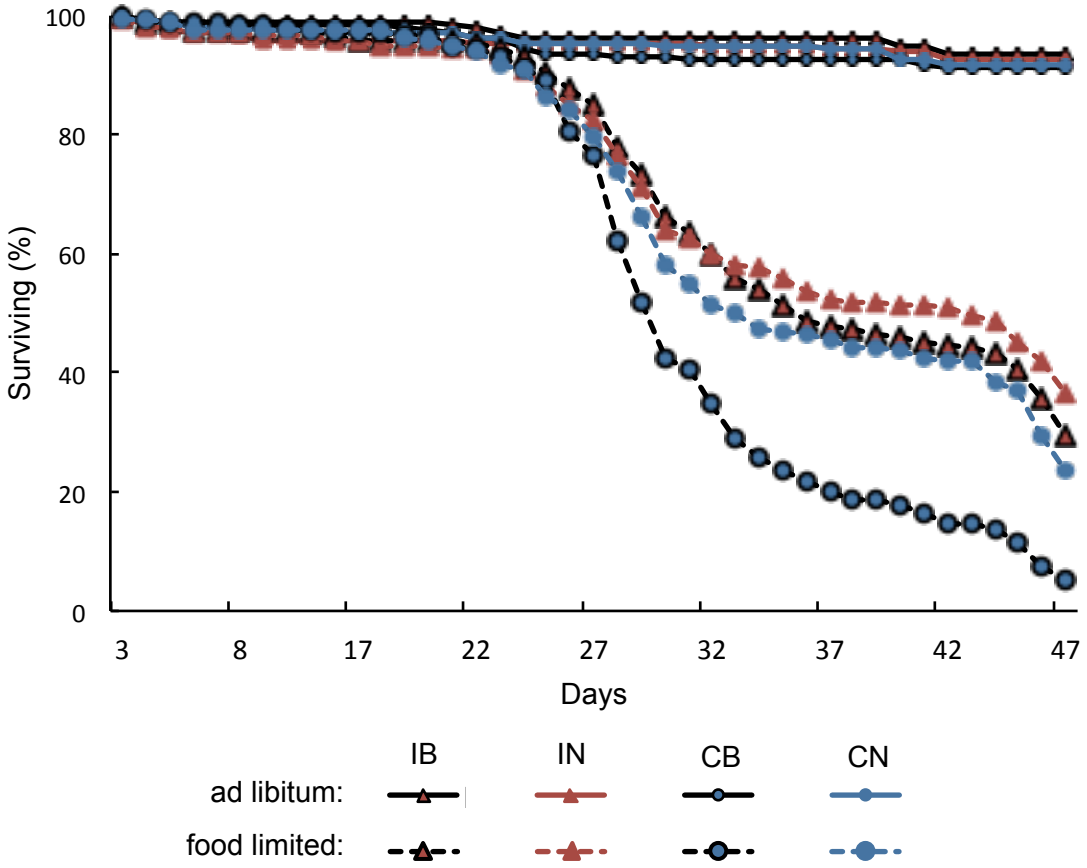


Figure 2.5. Survival to emergence for *M. c. pluviale* parents (n = 1599). Survival curves are based on raw data. IB = induced foliage with bacteria; IN = induced foliage without bacteria (naïve); CB = control foliage with bacteria; CN = control foliage without bacteria (naïve).

Fecundity

i) Food-limited treatments

Females fed induced foliage laid approximately 15% *more* eggs than those reared on control foliage (induced: 89 ± 5.6 eggs; control: 64 ± 8.5 eggs; $F_{1,14}$ = 5.887, P = 0.029). There was no effect of leaf microbiota on fecundity (bacteria: 73 ± 8.5 eggs; naïve: 80 ± 6.1 eggs; $F_{1,14}$ = 0.376, P = 0.550), and no interaction between the treatments (induction*microbiota: $F_{1,13}$ = 0.604, P = 0.451). Pupal mass was a significant

predictor of total egg count, with heavier females laying more eggs (y = -36.2 + 0.5*x; R^2 = 0.56; $F_{1,14}$ = 21.672, P < 0.001).

ii) Ad libitum treatments

Neither plant induction (induced: 223 ± 2.2 eggs; control: 220 ± 2.7 eggs; $F_{1,294}$ = 0.149, P = 0.700) nor leaf microbiota (bacteria: 223 ± 2.6 eggs; naïve: 220 ± 2.4 eggs; $F_{1,294}$ = 0.574, P = 0.449: induction*microbiota: $F_{1,293}$ = 0.119, P = 0.731) affected fecundity. Pupal mass was positively correlated with number of eggs laid (y = 71.4 + 0.3*x; R^2 = 0.22; $F_{1,294}$ = 101.232, P < 0.001).

Egg size and composition (ad libitum only)

Eggs dissected from ad libitum virgin females were analyzed for size, protein content, lipid content, and antibacterial activity.

i) Egg size

Eggs laid by females fed induced foliage were smaller than eggs laid by females fed control foliage ($F_{1,117}$ = 5.679, P = 0.018), but bacterial priming had no effect on egg size ($F_{1,117}$ = 0.665, P = 0.416) (Fig. 2.7a). Although Fig. 2.7a appears to indicate a trend for an interaction between plant induction and leaf microbiota, it did not reach statistical significance ($F_{1,116}$ = 2.461, P = 0.119) (Fig. 2.7a). A weak negative trade-off occurred between the number of eggs laid by a female and individual egg volume ($F_{1,118.3}$ = 20.656, P < 0.001; bivariate fit of egg volume by number: y = 0.64 - 0.0005*x; R^2 = 0.09), but there were no interactions between number of eggs, plant induction, and leaf microbiota (induction*egg number: $F_{1,116}$ = 1.192, P = 0.277; microbiota*egg number: $F_{1,117.4}$ = 0.101, P = 0.751; induction*microbiota*egg number: $F_{1,116.3}$ = 0.128, P = 0.721).

ii) Egg protein content

Egg protein content (ng per egg) was higher in eggs laid by females fed bacteria foliage compared to naïve foliage ($F_{1,93}$ = 5.301, P = 0.024) (Fig. 2.7b), but was not affected by plant induction ($F_{1,93}$ = 0.315, P = 0.576), and there was no interaction

(induction*microbiota: $F_{1,92}$ = 0.352, P = 0.554). Egg protein content was unrelated to female pupal mass ($F_{1,91}$ = 0.081, P = 0.776).

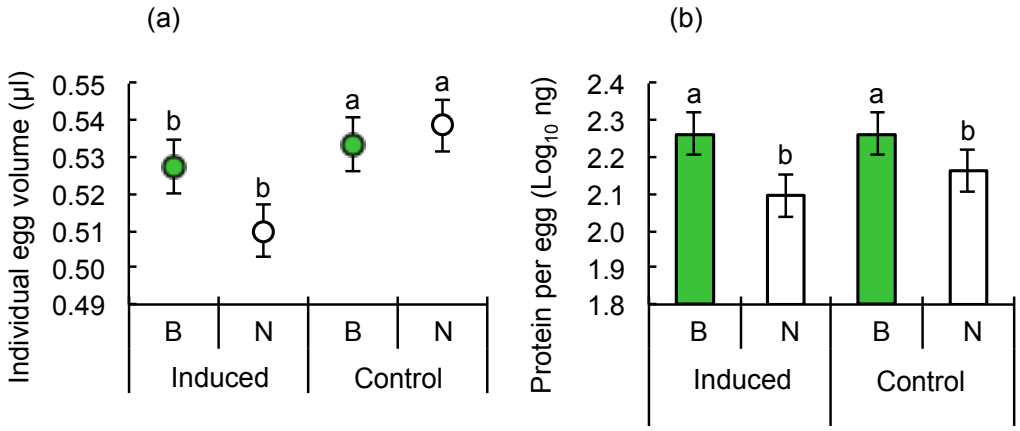


Figure 2.6. (a) Volume (n = 2,638) and (b) protein content (n = 96) of eggs produced by ad libitum female *M. c. pluviale*. For mean (± 1 SE) egg volume, female ID was included as a random effect to account for variation within egg batches (standard least squares, REML), while egg protein measurements are shown as LS means (± 1 SE) over a subsample of eggs (standard least squares). Protein levels are shown on a per egg basis, which was adjusted for differences in egg volume. "Induced" refers to a diet of foliage from larvae-infested branches, while "Control" denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application). Lowercase letters indicate significant main effects from either induced foliage or application of foliar bacteria; shared letters indicate no difference between treatments.

ii) Egg lipid content

Egg lipid content (ng per egg) did not vary with plant induction (induced: 1.71 ± 0.018 ng; control: 1.73 ± 0.017 ng; $F_{1,97}$ = 0.607, P = 0.438) or leaf microbiota (bacteria: 1.73 ± 0.018 ng; naïve: 1.7 ± 0.018 ng; $F_{1,97}$ = 1.440, P = 0.233), nor was there an interaction between the two (induction*microbiota: $F_{1,96}$ = 0.206, P = 0.651). There was a slight positive relationship between lipid content per egg and female pupal mass (y = 1.497 + 0.437*x; R^2 = 0.04; $F_{1,97}$ = 3.991, P = 0.049).

iv) Egg antibacterial activity

Antibacterial activity of the eggs in assays against *M. luteus* did not vary with either plant induction (induced: 1.84 ± 0.036 ng; control: 1.87 ± 0.037 ng; $F_{1,102}$ = 0.381, P = 0.538), leaf microbiota (bacteria: 1.85 ± 0.037 ng; naïve: 1.87 ± 0.035 ng; $F_{1,102}$ = 0.252, P = 0.617), or an interaction between them (induction*microbiota: $F_{1,101}$ = 1.722, P = 0.193). Also no relationship existed between lysozyme like content and female pupal mass ($F_{1,100}$ = 0.458, P = 0.500). Eggs were also assayed against *E. coli*, but showed no anti-bacterial activity, although antibiotic (ampicillin) standards showed normal ranges of activity.

2.A.3. Parental generation discussion

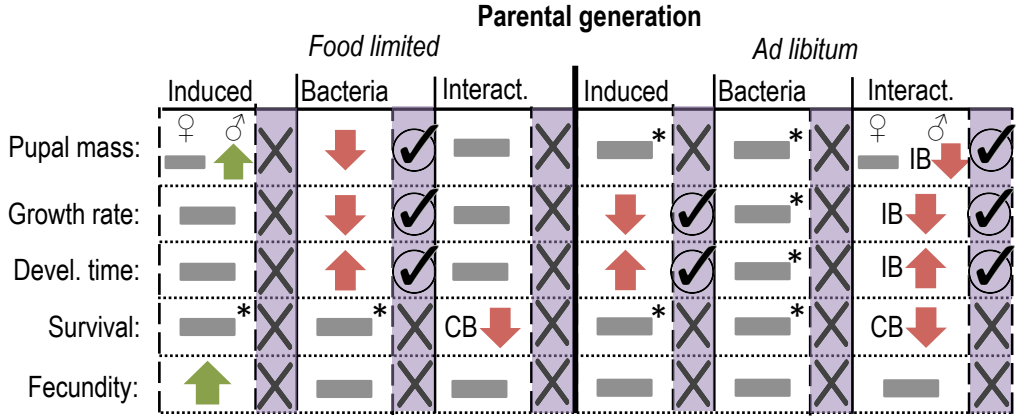
1) Parental life history traits

Parental life history characteristics were measured to assess the effectiveness of the treatments. We were interested in what costs or trade-offs might be evident in the parents, and how these potentially relate to differential provisioning of offspring. In general, we expected that the stressors would have negative effects on life history characteristics, and that combinations of stressors would amplify these negative effects, resulting in significant interaction terms. Specifically, we predicted that stressors would reduce pupal mass and growth rate, prolong development time, decrease fecundity, and reduce survival (in the absence of pathogen challenge) (Table 2.5). For reference, Table 2.6 gives a summary of effects in the parental generation as a function of plant induction and leaf microbiota.

Table 2.5. Predictions for single and multiple dietary stressors on the parental generation of *M. c. pluviale*. Red arrows pointing downward indicate negative effects; the number of arrows indicates the effect strength.

Parameters	Impact of stressors		Reasoning
	single	multiple	
Pupal mass, growth rate, fecundity, and survival	↓	↓↓↓	Stressors impose costs on life history parameters; multiple stressors act synergistically to decrease fitness.

Table 2.6. Summary effects of plant induction and foliar bacteria on the parental generation of *M. c. pluviale* as a function of food quantity. Arrows indicate the direction (increase/decrease) and nature of effect (green = positive, red = negative). Gray bars indicate no effects. Asterisks next to gray bars indicate presence of interactions, which are identified as treatments next to arrows (abbreviations: CB = control foliage and bacterially dosed; IB = induced foliage and bacterially dosed; Interact. = Interactions). Purple columns to the right of effects indicate whether results agreed with predictions; checks signify confirmations of predictions, while crosses indicate results contrary to predictions. Note that egg measurements are not included in this table.



Life history traits in the parental generation were negatively affected by food limitation across-the-board. These results are not surprising, and are consistent with previous studies regarding both variation in food quantity on insects (Donegan & Lighthart, 1989; Boggs & Freeman, 2005; Yang et al., 2007; Campero et al., 2008; Myers et al., 2011) and variation in nutrient content (Colasurdo et al., 2009; Triggs & Knell, 2012a). Overall survival was *much* lower when the larvae were reared on a food-

limited regime. This is considerably lower than in a previous study (Myers et al. 2011) in which 88% of food-limited larvae survived to pupation, compared to approximately 40% survival to pupation in this study. However, here the food limitation was applied during both the fourth and fifth instars as compared to only the fourth instar in the previous study. This extension of food limitation appeared to place too much stress on the insects at the sensitive pupation period when many died.

Although food limitation produced the largest effects, both plant induction and leaf microbiota modulated the impact of starvation. When starved insects were fed on foliar bacteria there were clear adverse consequences, including the exacerbation of negative effects on pupal mass, growth, male development time, and overall survival (although fecundity was unaffected). Negative effects from consuming bacteria were seen in both levels of food quantity, but why were they especially pronounced in starved insects? This may be related to the ability of insects to recoup costs of bacterial consumption. Freitak et al. (2007) showed that consumption of non-pathogenic bacteria (*E. coli* and *M. luteus*) in *T. ni* upregulated the immune response, resulting in costs to life history parameters. In starved or otherwise nutrient-deprived insects, immune activation can incur significant fitness costs, though such costs can be masked through compensatory feeding (Moret & Schmid-Hempel, 2000; Tyler et al., 2006; Valtonen et al., 2010). In a similar manner, I propose that food-limited larvae suffered more than ad libitum larvae from the ingestion of bacteria because they were unable to compensate for the incurred (presumably immune-related) cost. However, increased feeding would increase the intake of foliar bacteria. Such a relationship between consumption and microbial "dose" suggests that feeding ad libitum might confer greater costs from immune upregulation, although this association could be mediated by relative costs of immune activation (i.e. deployment) and maintenance (Lochmiller & Deerenberg, 2000; McKean et al., 2008; Valtonen et al., 2010).

Starved larvae fed non-induced foliage with bacteria had the lowest survival, which contradicts one of this study's main hypotheses (i.e. parents exposed to all three dietary stressors would fare the poorest all-around). However, this result agrees with direction of generally beneficial effects seen for food-limited larvae fed induced foliage: 1) survival to emergence was higher, 2) males were heavier, and 3) surviving starved

females had higher fecundity. One possible explanation for this pattern could be that herbivore damage resulted in foliage with a superior nutritional value (Das, 1968; Karban & Myers, 1989; Långström et al., 1990; Rooke & Bergström, 2007; Korpita et al., 2014). For example, Brown & Weis (1995) found that folivory by the leaf beetle *Trirahbda canadensis* on goldenrod, *Solidago missouriensis*, increased leaf flush, producing potentially more nutritious and preferred foliage. Haukioja et al. (1985) reported that leaf damage by the autumnal moth *Epirrita autumnata* on mountain birch *Betula pubescens* was correlated with higher foliar nitrogen content. However, among deciduous trees such as *A. rubra*, the response to herbivore feeding damage is generally associated with decreased leaf growth, foliar nutritional value, and potentially toxic allelochemicals (Nykänen and Koricheva, 2004; Schaller, 2008; but see Rooke & Bergström, 2006). Also, strong selection on starved insects is likely to have occurred and may have been important in determining successful pupation and stock of the next generation. Without a foliar analysis, it is difficult to predict what the true causes are for the differences within food-limited larvae as a consequence of foliar quality treatments.

In larvae given access to ample food, costs due to other stressors were clearly less severe than in starved larvae. Yet, even under ad libitum conditions, the combination of induced foliage with bacteria resulted in life history costs that surpassed the other ad libitum treatments: both sexes grew and developed more slowly and male pupae were smaller. Coors & De Meester (2008) tested the combined effects of pesticide exposure, predation threat, and parasitism on the waterflea *D. magna*. Even though food levels were high, combined stressors decreased immunocompetence and reduced population growth rate. In the honeybee *Apis mellifera*, insects challenged with LPS (lipopolysaccharide, an immune elicitor found in the membranes of Gram-negative bacteria) and fed a plant secondary compound (nicotine) showed decreased food consumption and survival versus non-stressed insects, despite an ad libitum food source (Köhler et al., 2012). Such studies suggest that life history costs may be more likely to be manifested in the presence of multiple stressors, despite ample resource availability. The interactive effects of induced foliage with bacteria are in contrast to the relative subtlety of such factors alone, which implies a non-additive effect.

The mild effects from bacterial exposure in otherwise non-stressed insects may seem surprising considering results reported by previous studies investigating the effects of non-pathogenic bacteria or immune elicitors on insects. A number of previous studies have sought to explore the nature of immune priming in insects through injection with bacterial suspensions (McKean & Nunney, 2005; Eleftherianos et al., 2006; Sadd & Schmid-Hempel, 2006; Linder & Promislow, 2009) or the immune elicitor LPS (Moret & Schmid-Hempel, 2000; Moret & Siva-Jothy, 2003; Tyler et al. 2006; Köhler et al., 2012). In general, these studies have highlighted how exposure to non-pathogenic bacteria or other immune elicitors can lead to fitness costs and immune activation in insects. However, immune challenge by injection bypasses the natural routes of entry, primarily via consumption, imposes an additional trauma by mechanical wounding (Vodovar et al., 2005; Freitak et al., 2007), and does not take into account the natural situation where the gut acts as both a barrier and reservoir for micro-organisms. There is a scarcity of studies detailing the life history consequences (or effects in general) of consuming non-pathogenic bacteria in insects. An exception is Freitak et al. (2007, 2009b) who were interested in how plant microbial communities affect herbivore fitness and immunity through incidental consumption. They found relatively strong effects from feeding on bacteria-dosed diets, both in terms of life history (Freitak et al., 2007) and gene expression (2009b). Their results, although novel, were based on what are likely to be unrealistically high (up to 40 – 400x higher for herbaceous or woody plants, respectively) dosages compared to the natural upper ranges for quantities of bacteria encountered by insects consuming foliage of either herbaceous or woody plants (Andrews et al., 1980; Stadler and Müller, 2000; Karamanoli et al., 2005; Enya et al., 2007; Yadav et al., 2008). In contrast, here I attempted to approximate natural levels of phylloplane bacteria, not exceeding the upper range found on actual leaf surfaces of deciduous trees (based on values from the literature). Additionally, the mode of application by Freitak et al. (2007, 2009a,b) was through soaking artificial diets with concentrated bacterial cultures, while our study applied bacterial suspensions through brief dipping of the leaves.

An alternative explanation for the relatively subtle effects from consumption of bacteria is that the application of these microorganisms to the leaf surface may have constituted a nutritional bonus for larvae, which could explain why otherwise non-stressed larvae were not strongly affected. There is evidence that mites (Hamilton et al.,

2003; Erban & Hubert, 2008), fruit flies (Drew et al., 1983), and other Dipterans (Lemos & Terra, 1991) can utilize bacteria as a food source, but I know of no such data for Lepidoptera. It is also important to note that Erban and Hubert (2008) tested bacterial digestion capability with a Gram-positive bacterium, *M. luteus*, and attributed this capability to the cleaving action of lysozyme. In contrast, the current study used Gram-negative bacterial species exclusively. As Gram-negative bacteria are far more resistant to enzymatic effects of lysozyme, it is less likely that lysozyme within WTC larvae could have effectively broken down bacteria and enabled them to utilize their nutritional value. In addition, if bacteria provided a nutrition boost to larvae, it may be expected that food-limited larvae would have benefited from bacteria on the leaf surface, as opposed to being clearly harmed by their presence.

The community composition and ecological significance of insect gut microbiota has recently received considerable interest (Hunt & Charnley, 1981; Dillon & Dillon, 2004; Indiragandhi et al., 2007; Dillon et al., 2010; Broderick et al., 2009; Indiragandhi et al., 2011), and it is likely that insects acquire many of their gut bacteria from environments such as the phylloplane (de Vries et al., 2001) or other plant parts (Kikuchi et al., 2007). Broderick et al. (2004) found that the gut bacterial community of gypsy moth *L. dispar* larvae fed on a range of host plants was composed of taxa commonly found on leaf surfaces and other environments, including *Pseudomonas* spp. and *Pantoea agglomerans*. Whether the bacteria used in this study could be considered "gut" bacteria in the WTC would require further research to determine their relative transience (versus indigenous microbiota) and their function in relation to insect development and physiology (Dillon & Dillon, 2004; Indiragandhi et al., 2011). However, some studies have suggested that gut bacteria are beneficial for insects (see reviews in Dillon & Dillon, 2004; Indiragandhi et al., 2011; Engel & Moran, 2013; but see Broderick et al., 2006; Ryu et al., 2008), while the current study again found only costs in the parental generation from dietary bacteria.

The overall effect of induced foliage on larvae fed ad libitum aligned with my predictions, as growth rate was decreased and development time was extended. Studies in the WTC have found negative effects from rapid induced resistance (RIR) on pupal mass (Rothman, 1997), growth rate (Adams, 1989; Sarfraz et al., 2013), and fecundity

(Sarfraz et al., 2013), though others have found no differences (Myers & Williams, 1984, 1987) or even beneficial effects (reduced developmental period – Rothman, 1997). In studies involving other forest Lepidoptera, RIR has been shown to have negative effects on pupal mass (Rossiter et al., 1988; Martemyanov et al., 2012), growth rate (Kapari et al., 2006), fecundity (Rossiter et al., 1988), survival (Haviola et al., 2007; Martemyanov et al., 2012), and prolong development time (Haukioja & Niemelä, 1979; Martemyanov et al., 2012). Although I predicted negative effects of induced foliage on pupal mass, this cost was not observed. The lack of an overall effect from induced foliage on pupal mass in ad libitum larvae could be partly due to the fact that insects were fed undamaged leaves from induced branches, while previous studies have not controlled for consumption of predamaged foliage (Rossiter et al., 1988; Rothman, 1997; Haviola et al., 2007; Martemyanov et al., 2012; although Sarfraz et al., 2013 did). Another explanation may lie in compensatory feeding (Simpson & Simpson, 1990; Raubenheimer & Simpson, 1993; Carisey & Bauce, 1997; Lavoie & Oberhauser, 2004), which could have allowed insects fed induced foliage to reach similar pupal weights compared to larvae fed control foliage. However, the compensatory ability of insects is not universal and it is unknown if the WTC is capable of such a strategy. Indeed, generalist herbivores (including the WTC), usually reach lower pupal weights from feeding on induced plant parts (Nykänen & Koricheva, 2004), while specialists are often able to successfully compensate for induced plant responses through increased feeding. The ability of insect herbivores to compensate can also be comprised when conditions involve chronic herbivory (Brown & Weis, 1995), high levels of allelochemicals (Wheeler, Slansky, & Yu, 2001), or nutritional imbalances (Lee et al., 2004).

Although the general effect of stressors on parental life history traits was negative and interactions between stressors usually intensified these negative effects, the results suggest that the outcome of multiple stressors is dependent on specific combinations and contextual background.

2) Egg size and quality

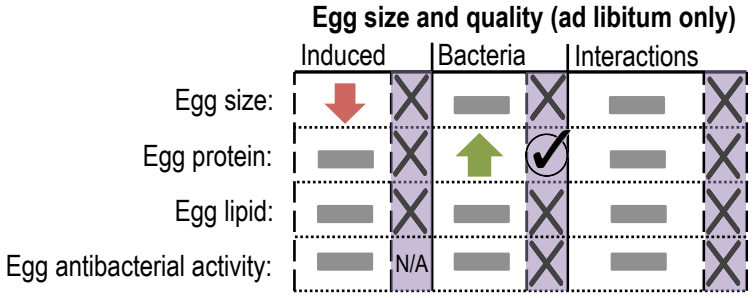
Maternal provisioning of offspring through eggs may play an important role as a potential mechanism for facilitating plasticity and adaptation to varying environmental

conditions across generations. Specific predictions regarding egg size and quality are outlined in Table 2.7, while a summary of results obtained is displayed in Table 2.8.

Table 2.7. **Predictions for effects of single and multiple dietary stressors on egg size and quality laid by moths of the parental generation of *M. c. pluviale*.** Red arrows pointing downward indicate negative effects; green arrows pointing upward indicate positive effects; the number of arrows indicates the effect strength.

Parental stressor	Egg size, proteins/lipids	Egg antibacterial activity	Reasoning
Food limitation	↑	?	Parents exposed to single stressors invest more into individual egg quality (at a cost to fecundity) in order to maximize offspring survival in the face of suboptimal environments. Parental exposure to bacteria enhances egg antibacterial activity. Multiple stressors overwhelm parents, leading to synergistic negative effects on egg provisioning.
Induced foliage	↑	?	
Foliar bacteria	↑	↑	
Multiple	↓↓↓	↓↓↓	

Table 2.8. **Summary effects of plant induction and foliar bacteria on size and quality of eggs taken from a subset of ad libitum moths of the parental generation of *M. c. pluviale*.** Arrows indicate the direction (increase/decrease) and nature of effect (green = positive, red = negative). Gray bars indicate no effects. Purple columns to the right of effects indicate whether results agreed with predictions; checks signify confirmations of predictions, crosses indicate results contrary to predictions, and "N/A" refers to relationships where no prediction was made.



Food-limited females clearly laid fewer eggs compared to females fed ad libitum but it was not possible to investigate any differences in egg size/number or composition due to constraints on sample size. Egg size was predicted to increase for females fed induced foliage or foliar bacteria, as I anticipated that these inputs would be "interpreted" by parents as stressors, meaning that parents might invest more into offspring quality versus quantity. Previous work with Lepidopterans suggests that stressful conditions associated with factors such as food limitation (Gliwicz & Guisande, 1992) and suboptimal diet quality (Torres-Vila & Rodrigues-Molina, 2002; Rotem et al., 2003) are generally correlated with larger eggs. Contrary to expectations, females fed induced foliage produced smaller eggs than those fed control foliage (but with no trade-off with fecundity) and the bacteria had no impact.

Contrary to expectations, eggs laid by females fed bacteria-dipped leaves did not have higher antibacterial activity than those treated naïvely. In Lepidoptera, Gorman et al. (2004) found upregulation in antibacterial activity after directly challenging eggs of the tobacco hornworm, *M. sexta*, with killed *E. coli* bacteria. In other insects, immune challenge with bacteria or LPS in the parental generation can enhance the antibacterial activity of eggs (Schmid-Hempel 2007; Moreau et al. 2012; Zanchi et al. 2012). Protein content was higher in eggs produced by females fed foliar bacteria, as predicted, though no difference existed for insects fed induced foliage. To my knowledge, this is the first study to show an effect on egg nutrient composition associated with the consumption of dietary bacteria. Although I showed that protein, lipid, and antibacterial activity did not differ in eggs as a function of maternal plant induction, it is possible that other unmeasured factors are important (e.g. amino acids (O'Brien et al., 2002) or antimicrobial peptides/immune-related proteins (Bettencourt et al., 1997; Faye & Kanost, 1998; Bettencourt et al., 2000; Terenius et al., 2007; Terenius, 2008; Zanchi et al., 2012)).

The effect that egg size has on overwintering survival of embryonic larvae, which is an important and extended period of the WTC developmental cycle, should also be considered. Some studies have suggested that larger insect eggs can correlate with increased survival when overwintering (Harvey, 1985; Gywnne, 1988; Fitzpatrick & Troubridge, 1993; Fischer, Brakefield, & Zwann, 2003), but the current study found no

difference between any of the offspring from ad libitum treatments in terms of neonate eclosion success (unpublished data). Although eggs were kept outdoors in near-ambient conditions, they were in a protected location and thus somewhat sheltered from severe weather conditions. During harsh winters or late springs, it is possible that egg size has an effect on overwintering survival in natural populations of the WTC.

2.B. Offspring generation

2.B.1. Offspring generation methods

To test for transgenerational effects of parental diet on offspring disease resistance and overall performance, a second generation of WTC larvae were reared in the lab on an ad libitum diet of red alder leaves. Offspring were assayed against McplNPV across a range of doses. In addition, a suite of immune related parameters and life history characteristics were measured to assess potential trade-offs and overall performance (see Figure 2.2 in the parental generation methods section for graphical representation of experimental design).

1) Offspring hatching and experimental setup

Overwintering egg masses were transferred to individually labeled 60 ml plastic cups at the end of March 2013 and kept in a protected location outside for approximately two weeks. Temperature generally varied between 8-14 °C, although on sunnier days temperatures reached as high as approximately 17 °C. Cups were checked regularly for larval eclosion.

On April 10, 2013, the first larval emergence was noted and the cups were transferred to an incubator at 10/5 °C on a 12/12 L/D schedule and this was gradually increased to 15/8 °C by early May to synchronize egg hatch and larval growth among families. Larvae were provided with fresh red alder leaves as needed. Leaves were washed with a 0.2% bleach solution for 5 mins, rinsed in tap water and air-dried before use. On May 9, larvae ranged from late 1st to early 2nd instars and were transferred into larger cups (473 ml waxed paper) and placed outdoors in a protected location so that

they could be reared at close to ambient temperature until used in the bioassays and other experimental procedures, at which point they were moved into the lab (approximately 20-24 °C).

For offspring of parents fed ad libitum, only families with at least 70 individuals were selected for use to ensure that adequate numbers of larvae would be available for all experimental procedures. Out of these "suitable" families, 20 per treatment were chosen at random to rear to the 4th instar.

Because survival was poor in the food-limited treatments, which resulted in far fewer egg masses (many of which were small), all families were retained for experimental use. Any families in either ad libitum or food-limited offspring with evidence of fungus, viral or other infections were discarded (n = 15).

Offspring used for life history analysis were weighed at the 4th instar, 5th instar, and as pupae.

2) Offspring virus assay

Offspring of ad libitum parents

The virus assay for offspring of ad libitum parents was set up in two blocks, one day apart. Newly moulted 4th instar larvae were weighed prior to administering sterile water (control) or one of five doses of purified *M. c. pluviale* NPV (the virus isolate was originally collected from a wild population and then amplified, see Cory & Myers, 2009). Virus doses were 5625, 11250, 22580, 45000, and 90000 OBs per individual. Virus doses were applied in 3 µl onto 0.8-cm-diameter red alder leaf discs and allowed to dry. Each leaf disc was placed on a moist piece of paper towel and placed in a 60 ml labeled plastic cup containing a pre-weighed larvae. Larvae were left to consume leaf discs for 24 h. Larvae that had eaten the leaf disc after this time were given fresh foliage. Larvae that had not finished leaf discs were given an additional 24 h to finish the disc. Larvae that had not eaten their leaf disc or had died during the 48 h period were discarded. Larvae were checked daily for mortality and date and cause of death were recorded for each individual. All larvae were provided fresh foliage daily and frass was cleaned out of

cups regularly. Two larvae per family per dose per treatment were used in each assay resulting in 960 larvae per block.

Offspring of food-limited parents

As fewer offspring were available from food-limited parents, only 1 dose (22580 OBs; plus an H_2O control group) were used and the numbers of individuals and families per treatment varied, depending on the availability of adequately sized families). This dose corresponded to the mid-range dose tested on offspring of ad libitum parents. Aside from this difference, the virus assay for progeny of food-limited larvae was carried out as described for offspring of ad libitum larvae.

3) Offspring immunity/condition measures

Larvae from the same families used in the virus assay involving ad libitum treatments were also used to measure a suite of immune parameters, namely, larval encapsulation ability, haemolymph phenoloxidase (PO) activity, protein concentration, antibacterial activity, and haemocyte counts. After use in the virus assay, two families developed disease (virus and fungus) and were not used for immunity measures.

Two subsets of larvae were used for immune parameters: 1) 4^{th} instars were bled to obtain haemolymph for PO activity, protein concentration, antibacterial activity, and haemocyte counts and 2) 5^{th} instars were used for measuring the encapsulation response.

Haemolymph measures

i) Bleeding and haemocyte counts

Fourth instar bleeding took place over two days. Each day, a single individual from each family (approximately 20/treatment), spread across the 4 treatments, was bled. Prior to bleeding, larvae were weighed, then immobilized on a piece of Parafilm (Bemis, Neenah, WI, USA) and pricked with a fine probe just above the left, terminal anterior proleg on the 6^{th} abdominal segment. A total of 7 µl haemolymph was pipetted directly from the wound and transferred to another piece of parafilm. Three µl of this

haemolymph were mixed with 60 µl of a phosphate buffer solution (0.2 M, pH 7) for PO/protein measurements. Another 2 µl of haemolymph was mixed with 10 µl phosphate buffer solution (0.2 M, pH 7) and 10 µl of this mixture was immediately pipetted into an improved Neubauer haemocytometer (VWR, Radnor, PN, USA). Haemocyte counts were done for the entire grid (400x magnification with phase-contrast illumination, using a Nikon H550L compound microscope). The remaining 2 µl of haemolymph, destined for measuring antibacterial activity, was pipetted into a 0.5 ml eppendorf tube containing a few crystals of phenylthiourea (PTU) (Sigma-Aldrich, St. Louis, MO, USA) to prevent haemolymph coagulation and melanization. Following collection, all haemolymph samples were frozen at -20 °C and transferred to -80 °C after several days.

ii) Phenoloxidase analysis

For PO analysis, samples were thawed on ice and centrifuged and vortexed briefly. Then 50 µl from each sample was pipetted into individual wells of a 96-well microplate kept on ice (see Shikano et al., 2010). Blanks of 0.17 M phosphate buffer solution were pipetted into wells alongside samples in each plate. Then, 150 µl of dopamine hydrochloride solution (11.3 mM in phosphate buffer) was added to each well. Absorbance was read at 492 nm every 45 seconds for 40 mins to obtain a kinetic activity record for enzyme activity. From these recordings, the enzymatic range as a function of absorbance was determined by calculating the Vmax for each sample, standard or blank. The remaining haemolymph/buffer solution was kept on ice for subsequent protein analysis.

iii) Protein analysis

Haemolymph protein was measured by the Bradford method (1976) and protocol followed the previous description given for protein analysis of eggs (see section 2.A.1).

iv) Antibacterial activity

Lytic zone assays were performed to evaluate the antibacterial activity of larval haemolymph. Protocol followed that given previously for analysis of antibacterial activity in eggs against *M. luteus* (see section 2.A.1).

No data were obtained for offspring haemolymph antibacterial activity, as no discernable inhibitory effect was observed on agar test plates of *M. luteus*, though standards produced consistent zones of inhibition (sensitivity of egg white lysozyme standards ranged from 0.125 – 1 mg/ml).

Encapsulation

A second subset of larvae was used to measure the encapsulation response in the 5th instar (done in 2 blocks, n = 78 per block, 2 larvae per family total). Larvae were weighed and then a small hole was made in the cuticle with a fine probe just above the left, terminal anterior proleg on the 6th abdominal segment. A small piece of nylon monofilament line (approximately 4 mm in length, 0.16 mm diameter, Sunko, Taiwan) was inserted into the hole. Nylon pieces were partially embedded in the haemocoel.

After implanting the nylon filament, larvae were transferred to individual 60 ml plastic cups, provided with fresh foliage, and left for 24 h. They were then frozen at -20 C for several days before filaments were removed. Nylon monofilaments were dissected from thawed caterpillars and placed into 1.5 ml eppendorf tubes filled with 70% ethanol for storage. Later, the nylon pieces were photographed under a dissecting scope (Nikon SM21500) using a camera (Nikon Digital Camera DXM1200F) at 15x and photographed twice, flipping 180° between shots. Photos were analyzed using ImageJ (1.47v, NIH, USA).

4) Statistical analysis

Fourth instar mass was initially included as a covariate in analyses of pupal mass, development time, and survival but initial mass was removed from final models if non-significant. Growth rate was estimated with the formula for relative growth rate (RGR) (Equation 1; see section 2.A.1).

Selection of final model terms was done by fitting all higher-order terms and removing non-significant terms sequentially. Significant interaction terms for general linear models were assessed with Tukey's HSD at $P < 0.05$. Interactions occurring with generalized linear models were explored through the use of contrast tests. Means, ±1

standard error, are given. Unless stated, statistics were performed using JMP (SAS Institute Inc. 2012), otherwise RStudio (0.97.310).

Values expressed in the results section (text, figures, or tables) are generally given as least square (LS) means, though some estimates are derived from GLM models or raw data, depending on the particular analysis.

All offspring parameters except mortality data were analyzed with family included as a random effect. Offspring mortality data were treated as a population level effect, and therefore family variation was not considered.

Susceptibility to McplNPV was analyzed with generalized linear models with a binomial logit link function. Data were right-censored 15 days after virus challenge. Initial mass and block were originally included as covariates, but were taken out of the final models if non-significant. Days to death from virus infection were analyzed using general linear models.

Transgenerational effects of food quantity

The effect of parental food quantity on offspring condition and disease resistance was analyzed as a single factor, excluding the effects of parental plant induction and leaf microbiota. Throughout ad libitum offspring analyses, data were analyzed in a factorial manner (2x2), incorporating effects of parental plant induction and leaf microbiota.

Sample sizes for family based comparisons were a major constraint as mated females from ad libitum treatments produced an average of 45.5 (+/- 1.8) viable egg masses per treatment, compared to food-limited treatments with far fewer female moths, which only produced an average of 4.5 (+/- 1.2) viable egg masses per treatment. In one food-limited treatment, fed non-induced and bacteria foliage, only one viable egg mass was laid. It was therefore impossible to perform a fully factorial (2x2x2) analysis of parental effects on offspring. Due to a difference in the set up dates of rear-through larvae in offspring of parents fed ad libitum vs. food limited, growth rate and development time (both highly dependent on developmental stage) could not be analyzed on the basis of parental food quantity.

Offspring of moths fed ad libitum as larvae were compared with offspring of food-limited larvae within a single virus dose (22,580 OBs), due to sample size constraints within offspring of food-limited parents.

Transgenerational effects of plant induction and leaf microbiota

The virus assay for offspring of ad libitum parents was initially fit with models incorporating fully factorial effects of plant induction x leaf microbiota x virus dose. Initial mass was not significant and was discounted from further analysis and models were based on treatment totals.

Total virus deaths were adjusted for any virus and background mortality in the untreated control insects. Adjusted virus mortality was calculated according to Equation 2:

Equation 2. $V_a = V_d - T_d * (V_c / T_c)$

Where V_a is the adjusted virus mortality, V_d is the dose-specific number dead by virus, T_d is the dose-specific number assayed, V_c is the number dead by virus in the untreated control dose, and T_c is the total number assayed for the untreated control dose.

Next, adjusted totals were calculated according to Equation 3:

Equation 3. $T_{adj} = T_d * (1 - (V_c + U_c) / T_c)$

Where T_{adj} is the adjusted dose-specific total number assayed for the analysis, and U_c is the number dead by unknown causes for the untreated control dose.

Median lethal dose (LD 50) values and confidence intervals for virus mortality were estimated using JMP by obtaining inverse predictions of GLM models at a probability of 0.5. Time to death from virus infection was analyzed with a general linear model. To look for differences related to sublethal exposure to virus, adult emergence was quantified for survivors (those larvae that pupated) in the virus assay, with initial model effects for the GLM including parental plant induction, parental leaf microbiota, and virus dose in a fully factorial design and offspring initial mass as a covariate.

Additionally, sublethal effects of virus on offspring pupal mass were assessed within several sets of larvae used in the virus assays: A) Between offspring of food-limited and ad libitum moths, looking for any differences between control and dosed larvae, B) Between offspring of food-limited and ad libitum moths, looking at potential differences within dosed larvae only, C) Within offspring of ad libitum moths, looking for any differences between control and dosed larvae, D) Within offspring of ad libitum moths, looking at potential differences within dosed larvae only. In all analyses, initial (4^{th} instar) mass was fit as a covariate and family was treated as a random effect. For within offspring of ad libitum moths, block was also included as a covariate in initial models.

Initial models for food-limited vs. ad libitum offspring were fit differently within each set. The analysis for set A (looking at control vs. dosed larvae) including fully factorial effects of parental food quantity and whether larvae were given the control treatment or virus dosed (control/dosed); sexes were analyzed separately due to bimodal distributions. Unlike set A, distributions of pupal mass based on sex in B (within dosed larvae only) were not bimodal and thus sex was included in a fully factorial design with parental food quantity. Set C incorporated a fully factorial design including plant induction, microbiota, and control/dosed. Set D was fully factorial for induction, microbiota, and $\log_{10}$ dose.

For offspring immunity measures and life history analyses, general linear models were used to investigate transgenerational effects of plant induction and leaf microbiota. For offspring immunity, mass at time of bleeding was included as a covariate, but was removed if non-significant. Phenoloxidase enzyme activities were $\log_{10}$ transformed, while haemocyte density was square root transformed to achieve normality.

Pupal mass and growth rates were analyzed separately by sex. Distributions of offspring pupal masses for both sexes were normalized by first reflecting data, followed by $\log_{10}$ transforming, and finally re-reflecting transformed values (Osborne, 2002). Female offspring growth rate was normalized through first adjusting the growth rate formula to include reflected values for pupal mass and initial mass, then re-reflecting growth rate values from the aforementioned calculation. Analysis of offspring

development time was done using a Cox Proportional Hazards mixed effects model, using the coxme (2.2-3) package in RStudio.

2.B.2. Offspring generation results

The low survival of food-limited parents and the resulting small number of egg masses produced made it impossible to analyze offspring results in a fully factorial manner (2x2x2). Instead, comparisons between offspring from different parental treatments were made by: 1) comparing the transgenerational effect of food quantity as a single factor (by lumping together treatments varying in plant induction and leaf microbiota), and 2) comparing the transgenerational effects of plant induction and leaf microbiota within offspring of ad libitum parents only.

Abbreviations and terms used in the description of the offspring generation results are identical to those used in the parental generation (see section 2.A.2).

1) Transgenerational effects of food quantity

Offspring disease resistance

When assayed with a single dose of NPV, offspring of food-limited parents were more resistant to NPV than offspring from parents fed ad libitum (Table 2.9a) and days to death between treatments was non-significant, with offspring from food-limited parents taking longer to die from viral infection compared to offspring of ad libitum larvae (Table 2.9a).

In contrast, background mortality within the group of virus-challenged larvae was higher for offspring of food-limited parents than those originating from ad libitum parents across a single dose (Table 2.9a). Background mortality did not differ between larvae dosed with virus or left untreated when comparing offspring of food-limited and ad libitum parents (dosed: 18.7 ± 2.2% mortality; untreated: 21.6 ± 2.32% mortality; likelihood ratio test: $\chi^2 = 0.835$, P = 0.361).

Offspring life history

Offspring survival to adult emergence for larvae was not affected by parental food quantity (Table 2.9b). Initial mass was negatively related to survival but the trend was very weak (χ^2 = 10.192, P = 0.001, R^2 = 0.01). Offspring pupal mass did not differ as a result of parental food quantity for females or males (Table 2.9b).

Table 2.9. Transgenerational effects of food quantity on (a) virus-induced mortality, background mortality, and speed of kill for McplNPV challenged 4th instars of the offspring generation and (b) survival to eclosion and pupal mass of offspring of *M. c. pluviale* parents. Estimates are based on raw averages (± 1 SE) for all parameters except those for infection duration, which is given as LS means (± 1 SE). P values in bold represent statistically significant effects at P<0.05.

a) Virus assayed larvae

Parameter	Food limited ± 1 SE (n)	Ad libitum ± 1 SE (n)	Statistics
Virus mortality*	25.5 ± 3.5% (157)	40.2 ± 2.8% (311)	χ^2=10.185 , **P=0.001**
Other deaths*	28 ± 3.6% (157)	19.6 ± 2.3% (311)	χ^2=4.141, **P=0.042**
Infection duration†	10.4 ± 0.4 days (40)	9.6 ± 0.2 days (125)	$F_{1,163}$=3.809, **P=0.053**

Deleted terms: Virus mortality, initial mass: χ^2=0.927, P=0.336; virus time to death, initial mass: $F_{1,162}$=1.336, P=0.249.

b) Larvae reared to eclosion

Parameter	Food limited ± 1 SE (n)	Ad libitum ± 1 SE (n)	Statistics
Surv. to emergence*	57.8 ± 3.1% (228)	54.3 ± 1.9% (547)	χ^2=0.094, P=0.760
♀ Pupal mass†	448.7 ± 5.7 mg (81)	456.4 mg (255)	$F_{1,53.78}$=0.864, P=0.357
♂ Pupal mass†	253.6 ± 4.0 mg (76)	254 ± 3.1 mg (138)	$F_{1,38.77}$=0.058, P=0.811

Deleted terms: Background mortality, initial mass: χ^2=0.564, P=0.453; ♀ pupal mass, initial mass: $F_{1,330.2}$<0.001, P=0.999; ♂ pupal mass, initial mass: $F_{1,193.1}$=0.069, P=0.793.

*Statistics reported from generalized linear model (GLM)
†Statistics reported from general linear mixed models

2) Transgenerational effects of plant induction and leaf microbiota (ad libitum only)

Offspring disease resistance

Plant induction had a strong transgenerational effect on offspring disease resistance, with offspring from parents fed induced foliage being less susceptible to NPV than offspring of parents reared on control foliage (χ^2 = 31.796, P < 0.001) (Fig. 2.8). Offspring from parents reared on foliage with bacteria also were significantly less susceptible to virus compared to offspring from parents fed naïve foliage (χ^2 = 4.135, P = 0.042) (Fig. 2.8; Fig. 2.9), with no interaction between treatments (induction*microbiota: χ^2 = 2.398, P = 0.122). Virus-induced mortality increased with increasing virus dose (χ^2 = 107.077, P < 0.001) (Fig. 2.9). The day the assay was set up significantly affected virus mortality (χ^2 = 9.044, P = 0.003), with overall mortality being lower in the second of two blocks. This was expected as larvae were slightly older (1 day) in the second assay.

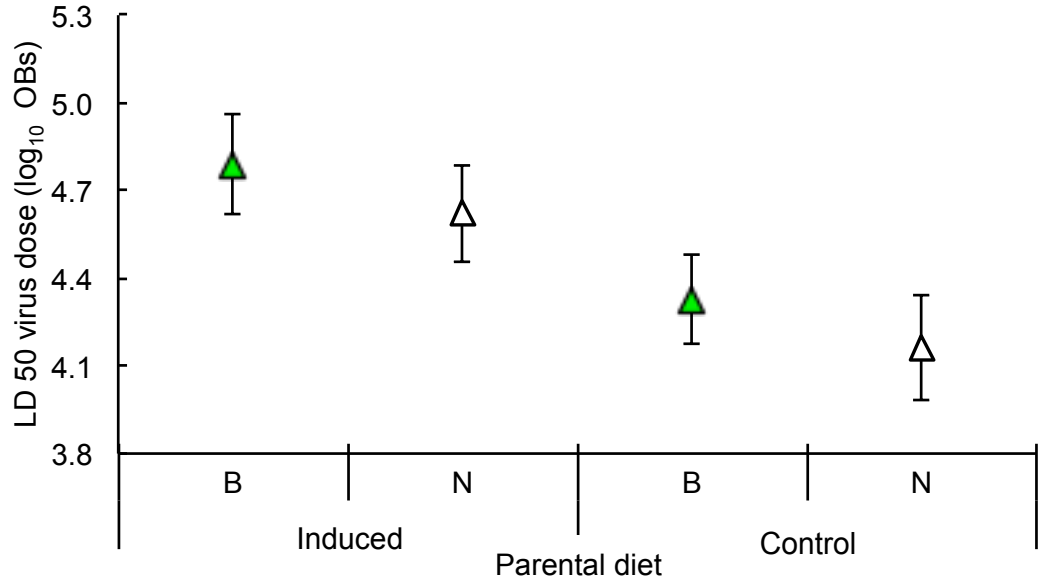


Figure 2.7. LD 50 for 4th instar *M. c. pluviale* offspring challenged with McplNPV (progeny of ad libitum only) (n = 1400). Variance of estimates is represented by 95% confidence intervals. "Induced" refers to a parental diet of foliage from larvae-infested branches, while "Control" denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

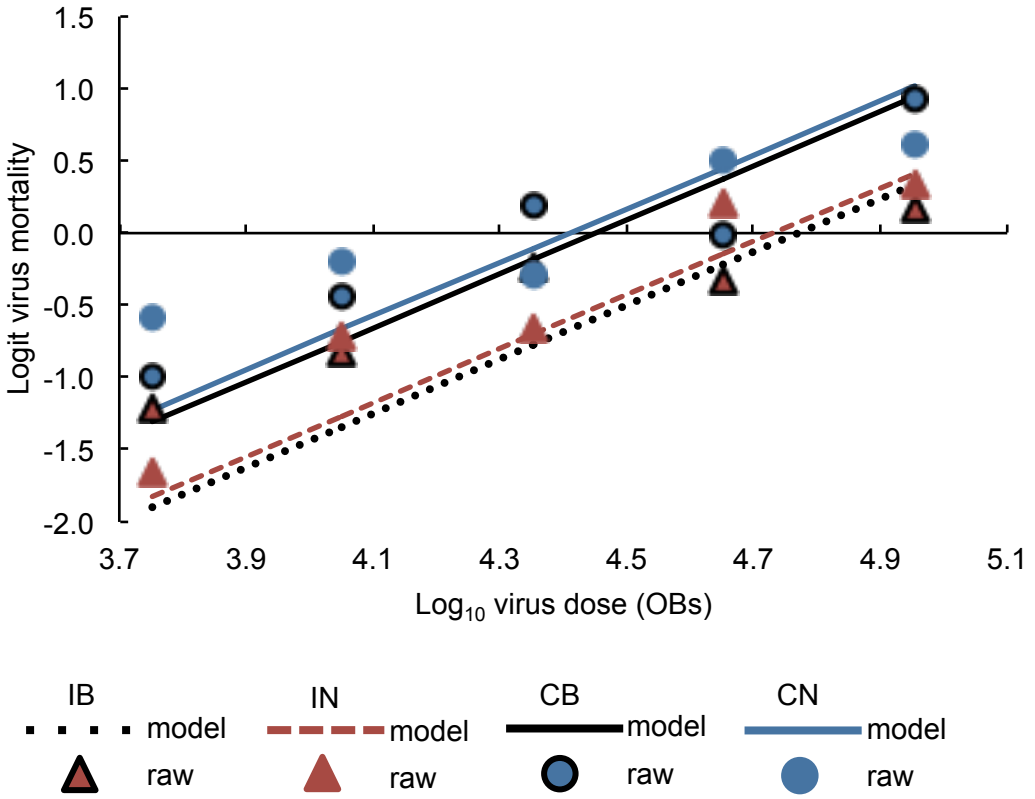


Figure 2.8. Variation in virus mortality of *M. c. pluviale* offspring of ad libitum parents (n = 1400). Raw data points are plotted with fitted model lines. Parental diet abbreviations: IB = induced foliage with bacteria; IN = induced foliage without bacteria (naïve); CB = control foliage with bacteria; CN = control foliage without bacteria (naïve).

Time to death from virus infection

Time to death from virus infection was influenced by both parental plant induction and leaf microbiota, but there was no interaction between the two (Fig. 2.10a; Table 2.10). Progeny of parents fed induced foliage took longer to die from viral infection than those fed control foliage, whereas larvae of parents fed bacteria treated leaves died more quickly from viral infection (Fig. 2.10a; Table 2.10). The interaction between leaf microbiota and virus dose was significant (Fig. 2.10b; Table 2.10); with offspring from parents reared on bacteria foliage taking longer to die as virus dose increased. This pattern was not observed for naïve treatments (Fig. 2.10b).

Table 2.10. Final statistical model for time to death from virus infection for 4th instar *M. c. pluviale* offspring of ad libitum parents challenged with McplNPV. Statistics are based on standard least squares models. P values in bold represent statistically significant effects at P<0.05. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	*F*	P
Induction	1	4.456	**0.035**
Microbiota	1	5.514	**0.019**
Log_{10} dose	1	0.526	0.469
Microbiota*Log_{10} dose	1	4.940	**0.027**
Error	613		

Deleted terms: Block: $F_{1,612}$=1.017, P=0.314; initial mass: $F_{1,611}$=0.418, P=0.518; induction*microbiota: $F_{1,610}$=2.402, P=0.122; induction*log_{10} dose: $F_{1,609}$=0.287, P=0.592; induction*microbiota*log_{10} dose: $F_{1,608}$=0.168, P=0.682.

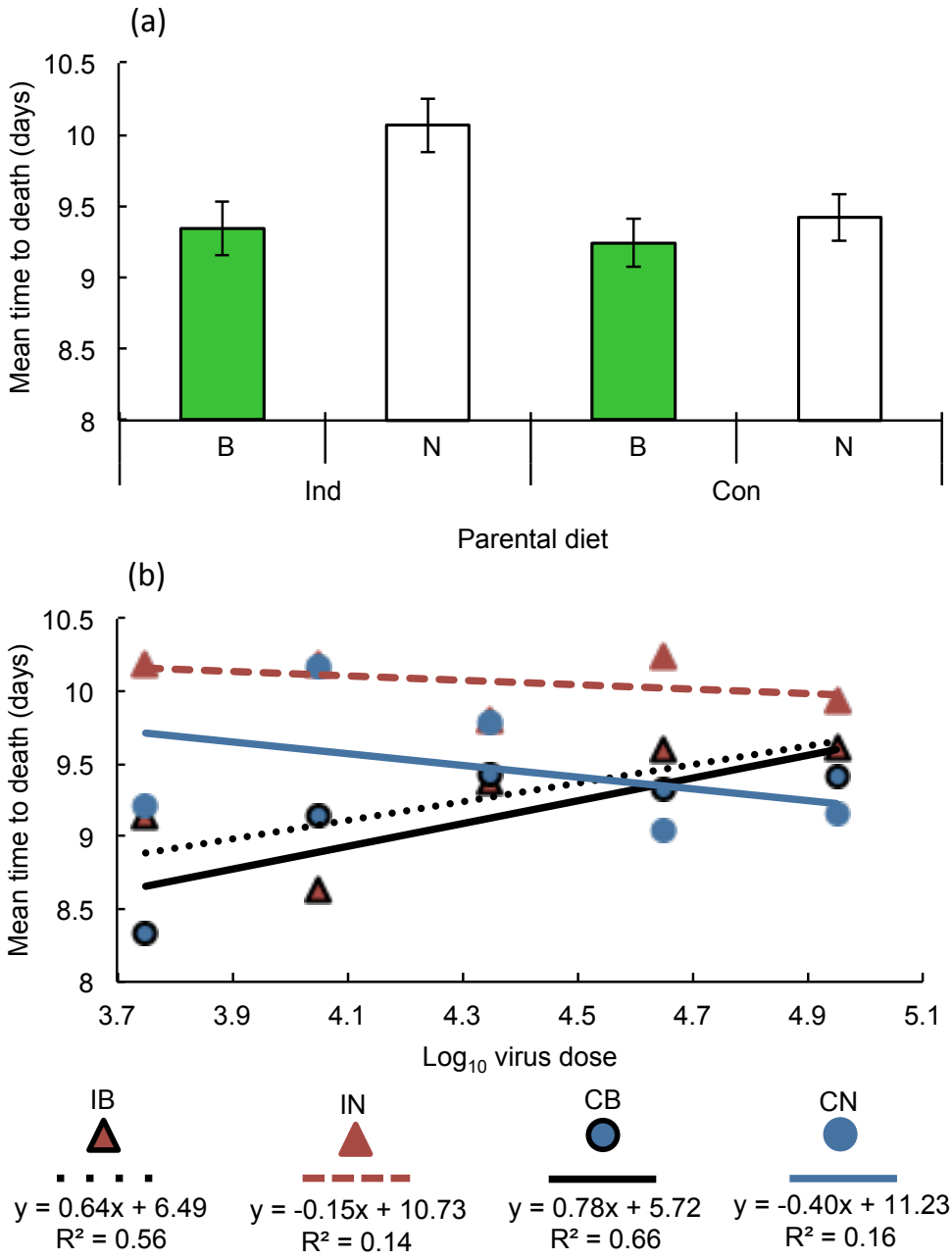


Figure 2.9. Time to death for 4th instar *M. c. pluviale* offspring of ad libitum parents (n = 647) challenged with McpINPV, showing mean time to death by (a) treatment, and (b) as a function of treatment and virus dose. Values in (a) are given as LS means (± 1 SE), while those in (b) are raw data plotted as points and trendlines. "Induced" refers to a parental diet of foliage from larvae-infested branches, while "Control" denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application). Parental diet abbreviations: IB = induced foliage with bacteria; IN = induced foliage without bacteria (naïve); CB = control foliage with bacteria; CN = control foliage without bacteria (naïve).

Background mortality in virus-challenged larvae

Background mortality averaged roughly 10% across all virus-challenged larvae but varied by treatment (Fig. A2; Table A2). Although there was no main effect of plant induction, offspring of parents fed bacteria foliage had lower mortality than offspring of parents fed naïve foliage, and there was an interaction between plant induction and leaf microbiota (Fig. A2; Table A2). Offspring of parents which were not exposed to any stress had the lowest survival: the induced foliage or bacteria (CN) treatment had the highest overall background mortality compared to IB (χ^2 =10.757, P = 0.001), IN (χ^2 = 9.627, P = 0.002), or CB (χ^2 = 28.817, P < 0.001) larvae (contrast tests; Fig. A2). In addition, fewer CB larvae died compared to IB (χ^2 = 4.549, P = 0.033) and IN (χ^2 = 5.283, P = 0.022) larvae (contrast tests; Fig. A2). Mortality due to background causes was not related to whether larvae were dosed with virus or water (dosed: 12.5 ± 2.1%; untreated: 10.3 ± 0.8%; likelihood ratio test: χ^2 = 1.145, P = 0.285).

Eclosion of virus-challenged survivors

Of the insects that survived to pupation, parental plant induction did not affect adult emergence success, but offspring of parents fed bacteria foliage had a greater proportion of eclosed moths (Fig. 2.11; Table 2.11) with no interaction between plant induction and leaf microbiota (Table 2.11). Pupal eclosion decreased with increasing virus dose, and initial mass was positively correlated with emergence rates (Table 2.11).

Table 2.11. Final statistical model for pupal eclosion of McpINPV challenged *M. c. pluviale* offspring of ad libitum parents. Statistics were reported from a GLM (binomial, logit). P values in bold represent statistically significant effects at P<0.05. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	$\chi 2$	P
Induction	1	1.522	0.217
Microbiota	1	8.917	**0.003**
Log_{10} dose	1	4.451	**0.035**
Initial mass	1	5.793	**0.016**

Deleted terms: Block: χ^2=0.315, P=0.574; induction*log_{10} dose: χ^2=2.512, P=0.113; microbiota*log_{10} dose: χ^2=1.842, P=0.175; induction*microbiota: χ^2=1.155, P=0.283; induction*microbiota*log_{10} dose: χ^2=1.824, P=0.177.

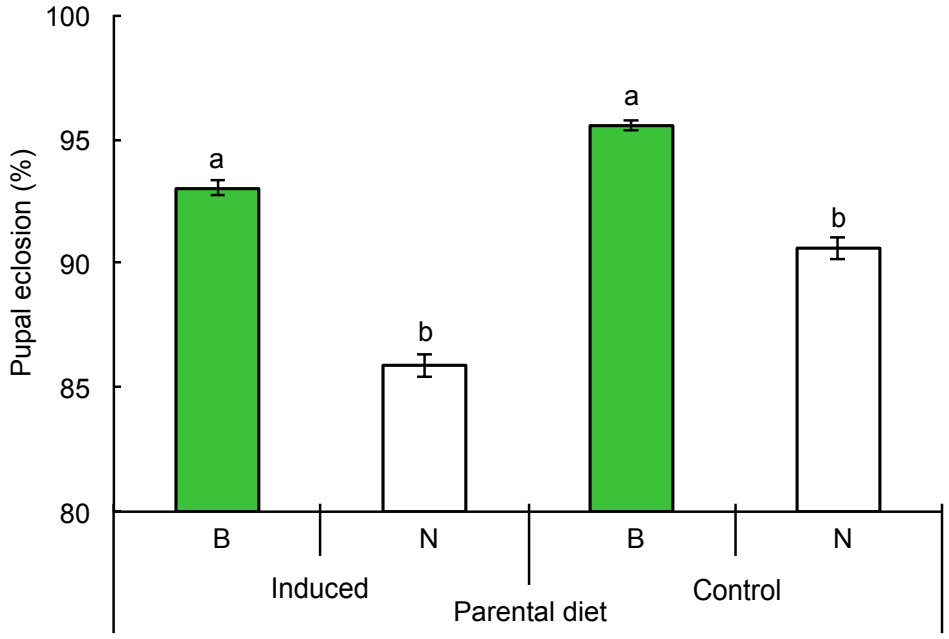


Figure 2.10. Pupal eclosion of survivors challenged with McpINPV for *M. c. pluviale* offspring of ad libitum parents (n = 526). Values are based on predicted GLM (binomial, logit) model values (± 1 SE) at P<0.05. "Induced" refers to a parental diet of foliage from larvae-infested branches, while "Control" denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application). Lowercase letters indicate significant main effects from application of foliar bacteria; shared letters indicate no difference between treatments.

Sublethal effects of virus challenge on pupal mass

i) Offspring of food-limited vs. ad libitum parents:

Pupal mass of virus challenged, female offspring of food-limited parents was lower than female progeny of ad libitum parents; male pupal mass was unaffected by parental food quantity (parental food quantity*offspring sex: $F_{1,149.5}$ = 6.179, P = 0.014; Fig. 2.12). Offspring of food-limited parents were lighter overall compared to offspring of ad libitum larvae ($F_{1,29.85}$ = 4.280, P = 0.047; Fig. 2.12) and females were heavier than males ($F_{1,149.5}$ = 731.961, P < 0.001; Fig. 2.12). Fourth instar mass was removed from the final analysis as it was a non-significant covariate ($F_{1,163.9}$ = 1.538, P = 0.217).

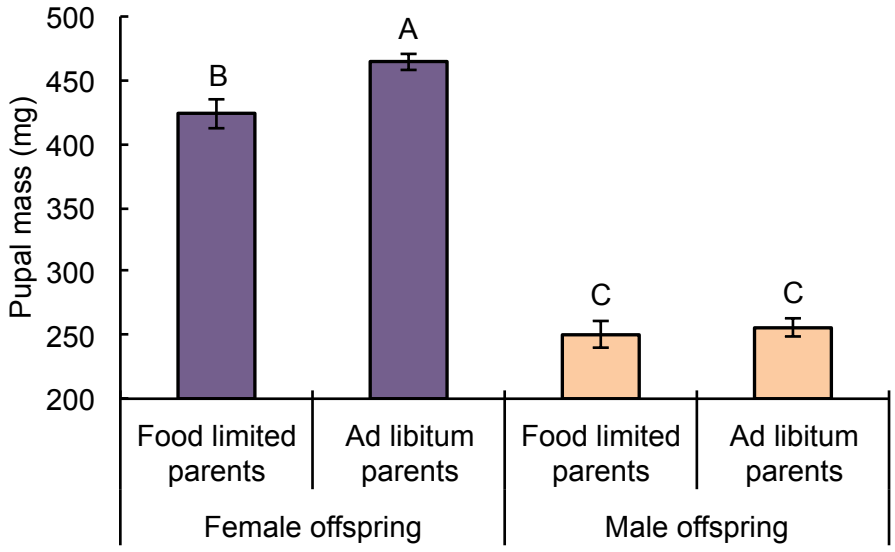


Figure 2.11. Sublethal effects of McplNPV challenge on *M. c. pluviale* offspring pupal mass as a function of sex and parental food quantity (n = 169). Figure depicts differences in larvae exposed to a single mid-level dose. Estimates are based on LS means (± 1 SE). Uppercase letters above bars represent a statistically significant interaction between treatment means (Tukey's test at P < 0.05). Levels with shared letters are not statistically different.

Within control treated and virus dosed larvae, females showed a non-significant trend for being lighter when parents were food limited compared to daughters of ad libitum parents (food limited: 1.415 ± 0.033 $\log_{10}$ mg [re-reflected]); ad libitum: 1.478 ± 0.015 $\log_{10}$ mg [re-reflected]), and there were no differences within males (food limited:

1.516 ± 0.043 $\log_{10}$ mg [re-reflected]; ad libitum: 1.562 ± 0.026 $\log_{10}$ mg [re-reflected]). For either sex, there were no indications of sublethal effects or other significant terms in the final models (Table A3).

ii) Within offspring of ad libitum parents:

There were no sublethal effects on pupal mass in any of the analyses for offspring of ad libitum treatments from the effects of virus treatment, dose, parental treatment, or any interactions for either offspring sex (Table A4, A5).

Offspring immunity and condition

i) Encapsulation

The encapsulation response of offspring was not influenced by main effects of plant induction ($F_{1,62.70}$ = 3.048, P = 0.086) or leaf microbiota ($F_{1,62.93}$ = 3.389, P = 0.070), but a parental diet of induced foliage without bacteria enhanced the encapsulation response in offspring (induction*microbiota: $F_{1,62.73}$ = 5.270, P = 0.025) (Fig. 2.13a). Larval mass at time of the encapsulation assay did not affect the melanization response ($F_{1,106.5}$ = 0.377, P = 0.540).

ii) Phenoloxidase activity

Offspring of parents fed induced foliage tended to have higher hemolymph PO activity, though this effect was borderline non-significant ($F_{1,66.52}$ = 3.431, P = 0.068), with offspring of parents fed induced foliage having higher PO activity levels compared to offspring from parents fed control foliage (Fig. 2.13b). Leaf microbiota did not influence PO activity ($F_{1,65.43}$ = 0.179, P = 0.673) and there was no interaction (induction*microbiota: $F_{1,65.24}$ = 0.003, P = 0.955). Mass at time of bleeding also approached significance and thus was included as a covariate in the final model ($F_{1,97.51}$ = 3.633, P = 0.060).

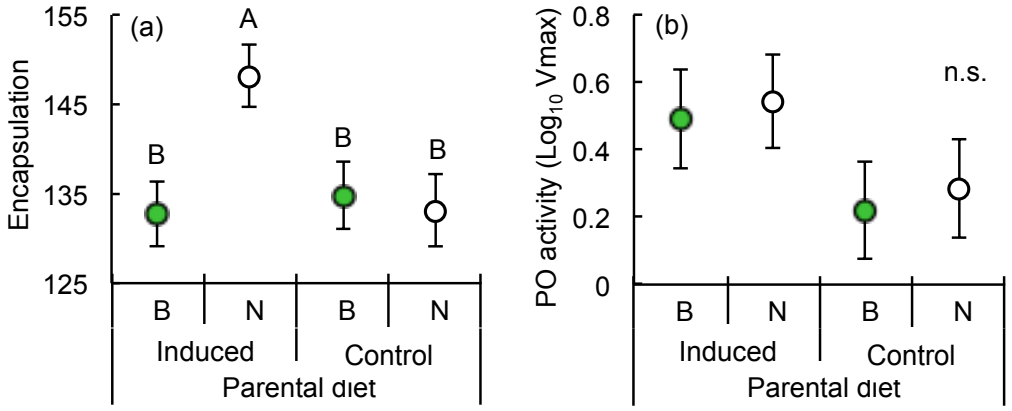


Figure 2.12. (a) Encapsulation response (n = 122), and (b) PO activity (n = 113) for 4th instar *M. c. pluviale* offspring of ad libitum parents. Values are given as LS means (± 1 SE) from standard least squares models. Uppercase letters above bars in (a) represent a statistically significant interaction between treatment means (Tukey's test at P<0.05); n.s.= non-significant. Treaments sharing letters are not statistically different. "Induced" refers to a parental diet of foliage from larvae-infested branches, while "Control" denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

iii) Haemocyte density

Offspring haemocyte concentrations did not differ in relation to parental food treatment (induced: 6.1 ± 0.21 √haemocytes/10 µl; control: 6.3 ± 0.21 √haemocytes/10 µl; $F_{1,76.02}$ = 0.402, P = 0.528), leaf microbiota (bacteria: 6.0 ± 0.21 √haemocytes/10 µl; naïve: 6.4 ± 0.21 √haemocytes/10 µl; $F_{1,76.03}$ = 2.070, P = 0.154), or any interaction between the two main effects (induction*microbiota: $F_{1,72.63}$ = 0.148, P = 0.702). Mass at time of bleeding did not affect larval haemocyte density ($F_{1,141.6}$ = 0.448, P = 0.505).

iv) Haemolymph protein

Offspring haemolymph protein did not vary with either parental plant induction (induced: 0.321 ± 0.009 mg/ml; control: 0.324 ± 0.009 mg/ml; $F_{1,75.35}$ = 0.064, P = 0.807), leaf microbiota (bacteria: 0.318 ± 0.009 mg/ml; naïve: 0.327 ± 0.009 mg/ml; $F_{1,71.77}$ = 0.507, P = 0.479), or an interaction between the two factors (induction*microbiota: $F_{1,71.03}$ = 0.043, P = 0.836). However, there was a strong positive correlation between larval mass at time of bleeding and haemolymph protein levels ($F_{1,116.1}$ = 74.838, P < 0.001).

Offspring life history

i) Survival

The survival of unchallenged offspring was enhanced when their parents were fed foliage with bacteria (χ^2 = 11.684, P < 0.001). There was no main effect of plant induction on survival (χ^2 = 0.899, P = 0.343), though there was a strong interaction (induction*microbiota: χ^2 = 25.604, P < 0.001; Fig. 2.14). Contrasts revealed that offspring of CN parents had poorer survival than offspring in any of the other treatments (compared to IB: χ^2 = 9.540, P = 0.002, IN χ^2 = 18.268, P < 0.001 and CB χ^2 = 35.531, P < 0.001). Additionally, offspring of IB larvae had lower survival compared to progeny of CB larvae (χ^2 = 8.354, P = 0.004). Initial mass did not influence survival to emergence (χ^2 = 0.232, P = 0.630).

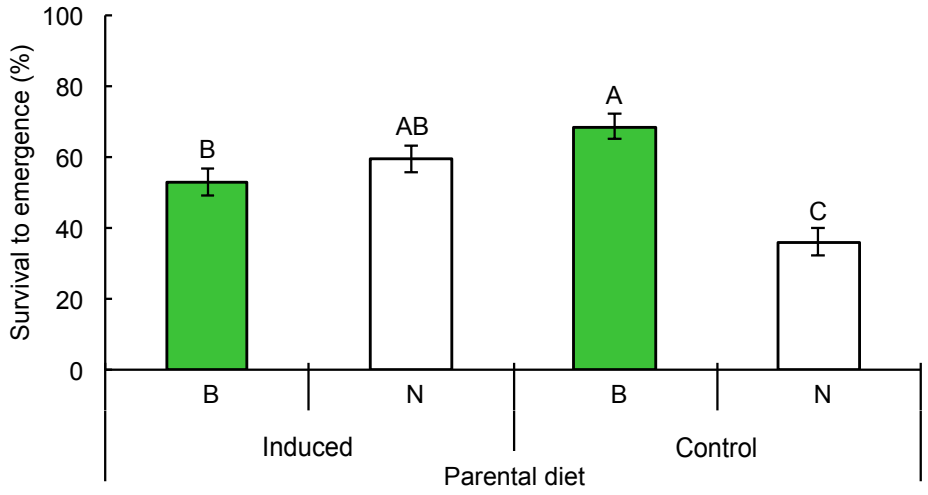


Figure 2.13. Survival to eclosion of unchallenged offspring of *M. c. pluviale* parents fed ad libitum (n = 653). Values are given as raw means (± 1 SE); statistics are based a GLM (binomial, logit). Uppercase letters above bars in (a) represent a statistically significant interaction between treatment means (contrast tests at P<0.05). Treaments sharing letters are not statistically different. “Induced” refers to a parental diet of foliage from larvae-infested branches, while “Control” denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

ii) Fourth instar mass

Fourth instar offspring of parents fed induced foliage were heavier than conspecifics from parents reared on control foliage ($F_{1,76.88}$ = 5.89, P = 0.018) (Fig. 2.15a). Offspring of parents fed bacteria foliage (60.4 ± 1.237 mg) showed a strong trend of being heavier compared to progeny of parents reared on naïve foliage (57.1 ± 1.241 mg) ($F_{1,76.88}$ = 3.698, P = 0.058) (Fig. 2.15a). There was no interaction between parental plant induction and leaf microbiota (induction*microbiota: $F_{1,75.91}$ = 0.127, P = 0.722).

iii) Fifth instar mass

At the 5th instar, the trend for the progeny of parents fed induced foliage towards being heavier than control foliage counterparts, continued but this was non-significant ($F_{1,68.72}$ = 3.129, P = 0.081) (Fig. 2.15b). Parental ingestion of bacteria did not affect 5th instar mass ($F_{1,66.38}$ = 1.394, P = 0.242), and there was no interaction between the two factors ($F_{1,65.91}$ = 0.649, P = 0.423). Initial (4th instar) mass was positively correlated with 5th instar mass ($F_{1,307.3}$ = 11.712, P < 0.001).

iv) Pupal mass

Pupal mass was lower in females from parents that were fed bacteria foliage ($F_{1,72.3}$ = 4.551, P = 0.036; Fig. 2.15c). Pupal mass of female offspring suggested a nearly significant difference due to transgenerational plant induction ($F_{1,72.4}$ = 3.932, P = 0.051), with offspring females from parents fed induced foliage being lighter than those from parents reared on control foliage (Fig. 2.15c). Although Fig. 2.15c visually suggests that offspring pupae from parents fed CN foliage were heavier compared to all other treatments, there was no statistically significant interaction between parental plant induction and leaf microbiota ($F_{1,73.5}$ = 1.770, P = 0.188).

Unlike offspring females, male pupal mass (n = 137) was unaffected by parental plant induction (induced: 256.4 ± 3.93 mg; control: 250.9 ± 4.83 mg; $F_{1,31.3}$ = 0.210, P = 0.650), leaf microbiota (bacteria: 251.7 ± 4.23 mg; naïve: 256 ± 4.67 mg; $F_{1,31.3}$ = 0.535, P = 0.470) or an interaction between the two factors (induction*microbiota: $F_{1,27.63}$ =

0.288, P = 0.596). Neither female or male pupal mass was affected by initial 4th instar mass (females: $F_{1,241.7}$ = 0.885, P = 0.348; males: $F_{1,125.8}$ = 0.046, P = 0.830).

v) Growth rate and development time

Growth rate of female offspring from full food treatments was lower when the parents were fed on induced foliage ($F_{1,57.77}$ = 7.967, P = 0.007; Fig. 2.15d). Bacterial priming did not affect female growth rate, though there was a slight non-significant trend, which suggested that bacterial exposure may have a slight negative effect ($F_{1,57.6}$ = 3.002, P = 0.089; Fig. 2.15d). There was no interaction between plant induction and leaf microbiota (induction*microbiota: $F_{1,59.55}$ = 2.385, P = 0.128).

Growth rate of male offspring (n = 137) was not affected by either treatment (induced: 0.117 ± 0.003; control: 0.119 ± 0.003; $F_{1,48.96}$ = 0.132, P = 0.718; leaf microbiota (bacteria: 0.117 ± 0.003; naïve: 0.119 ± 0.003; $F_{1,49.37}$ = 0.179, P = 0.674; induction*microbiota: $F_{1,48.7}$ = 0.022, P = 0.884).

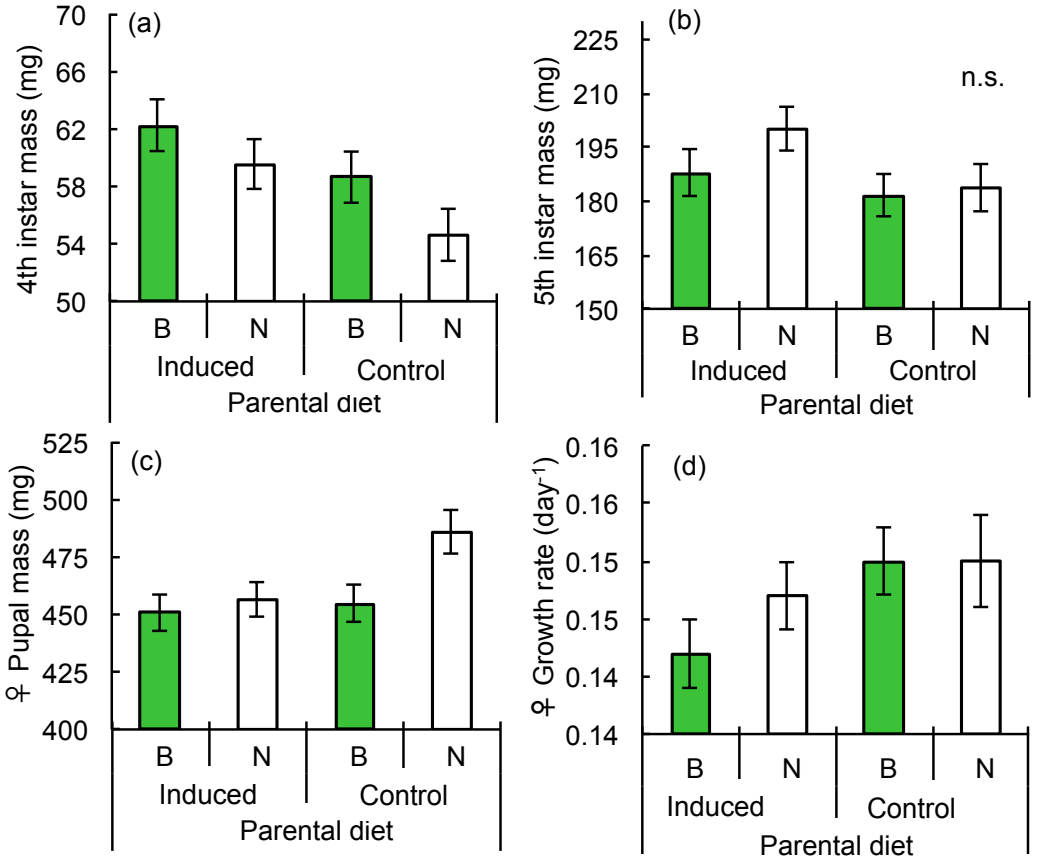


Figure 2.14. (a) Fourth instar mass (both sexes; n = 653), (b) fifth instar mass (both sexes; n = 324), (c) female pupal mass (n = 250), and (d) female growth rate (n = 250) for *M. c. pluviale* offspring of ad libitum parents. Fourth and 5th instar masses are given as LS means (± 1 SE), while female pupal mass and growth rate are given as raw means (± 1 SE). All parameters shown were analyzed with standard least squares models at $P<0.05$; n.s. = non-significant. "Induced" refers to a parental diet of foliage from larvae-infested branches, while "Control" denotes a parental diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

Development time was not influenced by parental plant induction, leaf microbiota, or an interaction between the two main effects (Table 2.12). However, there was a marginally non-significant trend for an interaction between leaf microbiota and sex which indicated that presence of bacteria in the diet of the parental generation reduced development times in male offspring, but female development time showed no effects (Fig. 2.16; Table 2.12). Offspring development time (4th instar – pupation) was affected by sex and initial mass; females took longer to develop compared to males and larvae

that were initially larger took less time to develop compared to smaller larvae (Table 2.12).

Table 2.12. Final statistical model for development time from 4th instar to pupation for *M. c. pluviale* offspring of ad libitum parents. Development time was assessed through a mixed effects Cox proportional hazards model. P values in bold represent statistically significant or marginally non-significant effects. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	Z	P
Induction	1	0.22	0.830
Microbiota	1	-1.62	0.110
Sex	1	-5.62	**<0.001**
Microbiota*sex	1	1.92	**0.055**
Initial mass	1	8.87	**<0.001**
Error	41.41		

Deleted terms: Induction*microbiota: $Z_{1,40.88}$=1.530, P=0.130; induction*sex: $Z_{1,41.45}$=-0.41, P=0.680; induction*microbiota*sex: $Z_{1,42.6}$=1.120, P=0.260.

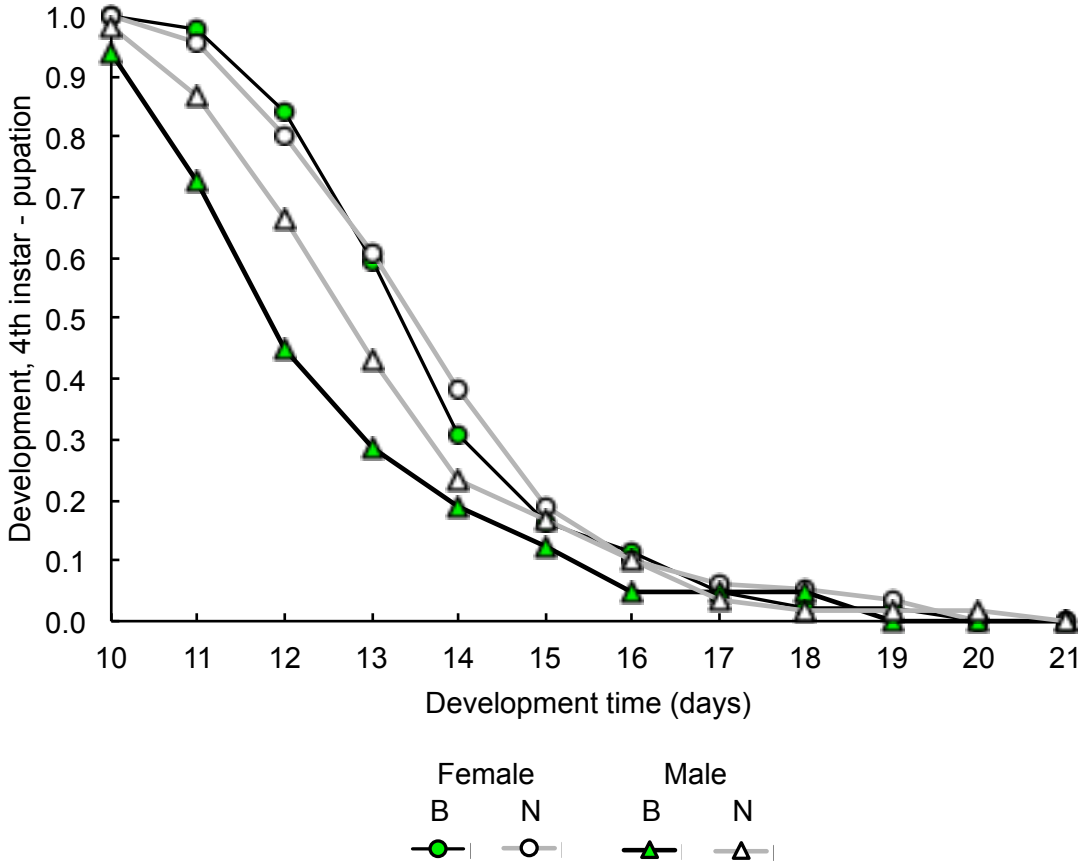


Figure 2.15. Proportion development from 4th instar to pupae plotted against days in rear-through larvae for *M. c. pluviale* offspring of ad libitum parents (n = 397). Development curves are based on raw data. B = bacterial application; N = naïve (without bacterial application).

2.B.3. Offspring generation discussion

Complex stressor-dependent costs in parents provided evidence for changes to life history and potential trade-offs, but how did these effects translate across generations? The results of this study clearly demonstrate that environmental factors can have a transgenerational impact on offspring disease resistance and immunity in the WTC. Specific predictions for the effect of parental treatments on the offspring generation are outlined in Table 2.13; a summary of results obtained for the offspring of ad libitum moths is presented in Table 2.14.

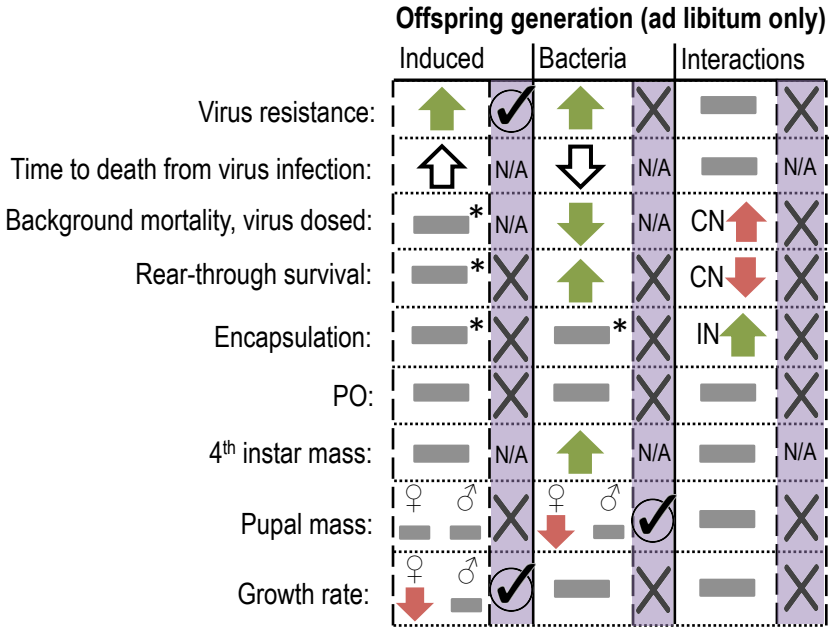
Table 2.13. Predictions of single and multiple parental dietary stressors on the offspring generation of *M. c. pluviale*. Red arrows pointing downward indicate negative effects; green arrows pointing upward indicate positive effects; the number of arrows indicates the effect strength.

Parental stressor	Virus resistance	Encapsulation, PO activity, and haemocyte count	Antibacterial activity	Pupal mass, growth rate, and survival
Food limitation	↑	↑	?	↓
Induced foliage	↑	↑	?	↓
Foliar bacteria	?	?	↑	↓
Multiple	↓↓↓	↓↓↓	↓↓↓	↓↓↓

Reasoning

Single stressors experienced by parents will upregulate offspring virus resistance and immunity at a cost to life history traits, though exposure to foliar bacteria in parents may result in higher virus mortality in offspring, as this constitutes a heterologous immune challenge between generations. Multiple parental stressors lead to inadequately provisioned offspring with negative synergistic effects on disease resistance, immunity, and overall fitness.

Table 2.14. Summary of transgenerational effects of plant induction and foliar bacteria on the offspring generation of *M. c. pluviale* within offspring of ad libitum insects. Arrows indicate the direction (increase/decrease) and nature of effect (green = positive, red = negative, white = N/A). Gray bars indicate no effects. Asterisks next to gray bars indicate presence of interactions, which are identified as treatments next to arrows (abbreviations: CN = control foliage and naïve (no bacteria); IN = induced foliage and naive). Purple columns to the right of effects indicate whether results agreed with predictions; checks signify confirmations of predictions, crosses indicate results contrary to predictions, and "N/A" refers to relationships where no prediction was made.



It was predicted that parents exposed to multiple stressors would produce progeny that were more susceptible to virus. However, presence of any of the three stressors (i.e. food limitation, induced foliage, and phylloplane bacteria) in the parental generation led to offspring that were more resistant to viral disease, while no interactive or non-additive effects on transgenerational disease resistance were evident (though we were unable to assess interactions within offspring of starved individuals).

Costs to parental and offspring life history (e.g. growth rate, pupal mass, fecundity) from all three parental diet factors were observed, suggesting that resource allocation within stressed individuals was shifted from growth (and in some cases overall

survival) to heightened immune function/disease resistance in offspring, though these shifts were not always uniform in direction.

1) Effects of parental food limitation on offspring: transgenerational effects or selection?

Offspring of food-limited parents showed greater resistance to NPV by approximately 15% higher survival over offspring of ad libitum larvae. The direction of this result agrees with an earlier study on WTC that suggested a trend towards increased offspring resistance to virus after parental food limitation (Myers et al. 2011). However, partial starvation decreased survival in both studies and it is not possible to distinguish between selection and/or transgenerational effects. Regardless, food limitation in the field would likely involve partial starvation leading to selection pressures that may be an important part of population cycles.

Few studies have explored the effects of parental food limitation on offspring disease resistance in invertebrates. Other than the WTC experiment by Myers et al. (2011) I am not aware of any studies that have investigated the effect of periodic starvation on disease resistance in Lepidoptera, though there are studies that have considered the effect of nutrient dilution with cellulose on transgenerational disease resistance in invertebrates. As cellulose has no nutritional value and is costly to process, such dilution could be considered tantamount to chronic, low-level starvation. Among Lepidoptera, evidence seems to favour enhanced virus resistance from a diluted diet for both the Indian meal moth, *Plodia interpunctella* (Boots & Roberts, 2012), and for the cabbage looper *T. ni* (Shikano et al., in press), although the presence of selective effects or separation from transgenerational effects were not reported in the former study. In other studies relating to "poor" diet quality, effects on disease resistance are more equivocal. In the common fruit fly, *Drosophila melanogaster*, a yeast-poor diet did not alter offspring resistance (survival) against a bacterial pathogen (Valtonen et al., 2012). In a non-insect example, waterflea (*Daphnia magna*) mothers that were given a dilute diet (a lower concentration of algal cells) produced offspring that were more resistant to bacterial disease compared to maternal diets with higher concentrations of algae (Mitchell & Read, 2005). However, studying the same species, Frost et al. (2010) found diets limited in phosphorus resulted in offspring that were more susceptible to the same

bacterial pathogen used in Mitchell & Read's (2005) study. This indicates that the *nature* of nutrient limitation may be essential in determining the direction or presence of transgenerational disease resistance.

Although parental food limitation appeared to play a protective role for virus-challenged offspring, offspring of starved insects were more likely to die from background causes compared to offspring of ad-libitum fed larvae, which possibly indicates a trade-off involved in increased immune response to virus at a cost of increased susceptibility to other pathogens (Little et al., 2003; Moret, 2006; Sadd & Schmid-Hempel, 2009). Alternatively, it is possible that insects that died of virus were also destined to die from background pathogens, but NPV infection overwhelmed other disease causing agents (Kaya & Koppenhöfer, 1996; Fuxa et al., 1999; Raymond et al., 2006). Therefore, the difference between virus and background mortality in offspring of food limited and ad libitum larvae could be the result of increased virus mortality in progeny of ad libitum offspring, leading to presumably fewer remaining larvae to be killed by other pathogens.

Pupae of female survivors of virus challenge were smaller when parents were food limited, which suggests that surviving virus infection is costly for offspring of starved insects. This is interesting because there was no such difference in pupal mass between offspring of food-limited and ad libitum insects in the absence of virus challenge. In the WTC, reduced fecundity and pupal mass from surviving NPV infection has been documented (Rothman & Myers, 1994, 1996a,b), and costs to females appear to be generally more evident than in males. These observations may account for fecundity declines following peak populations (Myers & Kuken, 1995; Cory & Myers, 2009). Although sublethal effects of baculovirus challenge have been shown as a result of low food levels in Lepidoptera (Boots & Begon, 1994; but see Myers et al. 2011), this is (to my knowledge) the first example of a sublethal effect apparently dependent on *parental* resource deficit.

Studies that have examined the transgenerational effects of nutrient-poor diets in insects have also demonstrated other impacts on life history, such as offspring weight (Valtonen et al., 2012; Triggs & Knell, 2012; Matzkin et al., 2013). In our study, offspring

pupal mass was unaffected by parental food limitation. Although this may seem counterintuitive, this result aligns with previous studies investigating transgenerational effects of starvation in insects (Fox et al. 1999, Saastamoinen et al. 2013; Frago & Bauce, 2014) and presumably may relate to selection. High mortality in food-limited parents may have selected for fitter individuals that were able to adequately provision offspring. We were not able to compare other life history traits between offspring of food-limited and ad libitum parents, which may have revealed trade-offs between pupal weight and growth patterns. For example, transgenerational food limitation in the spruce budworm, *Choristoneura fumiferana,* (Frago & Bauce, 2014) and the Glanville fritillary butterfly, *Melitaea cinxia*, (Saastamoinen et al. 2013) suggests that offspring pupal mass can remain unaffected by parental food limitation, although other life history parameters (e.g. growth and development time) were altered. As mentioned earlier, I suspect that starvation-induced selection pressures may have influenced our results, complicating the comparison between our study and that of Frago & Bauce (2014) and Saastamoinen et al. (2013). While Frago & Bauce (2014) found that food limitation over multiple generations significantly reduced survival, chronic food limitation only served to decrease fitness over generations, rather than selecting for fitter individuals. Saastamoinen et al. (2013) reported no negative impact on survival due to starvation, suggesting that selection played no role in their study either. While these studies suggest that parental starvation does not affect offspring pupal mass, it is not possible to separate the potential for selection from other effects in the current study.

2) Transgenerational effects of plant induction and leaf microbiota

The clearest result arising from this study was that offspring of parents reared on induced foliage were more resistant to viral infection compared to the progeny of parents fed control foliage. To my knowledge, this is the first evidence for a transgenerational effect on disease resistance or immunity from the consumption of induced foliage in an insect. Aside from two studies – one reporting no transgenerational effects due to plant induction (technically from high larval density on *A. rubra* trees) in the WTC (Rothman, 1997) and the other reporting significant effects on offspring life history in the gypsy moth, *L. dispar* (Rossiter, 1991 – discussed later) – I am not aware of other studies that have explored transgenerational effects of plant induction on insect herbivores in any capacity. The effect of parental consumption of foliar bacteria on offspring disease

resistance was not strong, and tended to increase resistance to virus. This result suggests a generality for transgenerational immune challenge and runs contrary to my original hypotheses based on other transgenerational immune priming (TGIP) studies. Interestingly, and despite the effect of parental bacteria on offspring disease resistance, no correlations were found between parental bacterial exposure and offspring immune response.

In the absence of virus challenge, survival was the lowest overall for offspring of parents fed with control foliage without bacteria (i.e. the “no-stress” treatment). This result is in contrast with the prediction that multiple parental stressors would lead to the lowest survival and highest levels of background disease in offspring, as this could be a potential mechanism for the triggering of general increased susceptibility to disease associated with poor larval condition in natural populations of the WTC. Interestingly, there was a slight suggestion that there might be some level of a transgenerational cost to combined stressors (versus single stressors), as offspring of parents fed induced foliage with bacteria (i.e. combined stressors) suffered a significant (~15%) decrease in survival versus progeny of insects fed control foliage with bacteria. However, the overall effect of parental bacterial consumption on offspring survival was positive, suggesting generalized protection against disease. Although offspring larvae in the current study were not experimentally immune challenged (aside from the separate cohort used for testing virus resistance), naturally occurring background disease other than NPV may be an additional or contributory source of mortality for WTC larvae at high densities (Cory & Myers, 2009; Sarfraz et al., 2013). In environments with high pathogen risk, such costs to survival in offspring may be offset. Roth et al. (2010) found that parental exposure (through injection) to bacteria improved offspring survival upon bacterial challenge, as compared to the progeny of naïve parents. I speculate that parental exposure to foliar bacteria somehow conferred general protection against background pathogens through upregulation of immunocompetence.

In order to begin to understand how induced responses in plants might act on herbivore disease resistance and immunity in a transgenerational manner, it is important to first review what is known about the general relationships between insect herbivores, induced plant defenses, entomopathogens, and insect disease resistance. The

combination of these factors is little studied and the few studies that have examined this tritrophic relationship have not found a consistent pattern (Cory & Hoover, 2006). One interesting aspect that complicates this relationship is the contradictory nature of herbivore-induced plant secondary chemicals, which both harm the performance of herbivores (possibly increasing susceptibility to infection) but also reduce the effectiveness of entomopathogens (Cory & Hoover, 2006; Pieterse & Dicke, 2007; De Roode, et al., 2011). For example, in the gypsy moth, *L. dispar*, feeding on induced foliage from red oak, *Quercus rubra*, led to chemical changes in foliage that negatively affected growth and reproduction (Rossiter, Schultz, & Baldwin, 1988), while at the same time these changes in secondary chemical content protected larvae from viral mortality (Hunter & Schultz, 1993). In the WTC, Rothman (1997) found that higher densities of larval colonies on *A. rubra* trees were more susceptible to NPV mortality, suggesting either that induced plant defences may be intimately linked with density-dependent virus transmission in the field, or that transmission was simply greater at higher density. In another WTC study, Sarfraz et al. (2013) reported lower mortality from unidentified pathogens in larvae fed foliage from herbivore-damaged branches of *A. rubra*, although resistance to baculovirus was unaffected.

The direct action of plant secondary chemicals (PSCs) in artificial diets on pathogen susceptibility has been studied in greater detail, but no consensus exists as to whether effects of PSCs on herbivore disease resistance are positive (Felton & Duffey, 1990; Young et al., 1995; Hoover, 1998; Martemyanov et al., 2006; Singer et al., 2009), negative (Cook et al., 2003), or null (Lindroth et al., 1999; Ali et al., 2002). These studies also vary in the timing of the putative PSC-pathogen interactions, ranging in three general temporospatial areas: 1) the study of mechanistic mid-gut level virus defence (Felton & Duffey, 1990; Hoover et al., 2000; Washburn et al., 1995, 1998; Keddie et al., 1989), more generally accomplished through PSC application with the pathogen at the time of infection (Lindroth et al., 1999; Cook et al., 2003), 2) rearing of insects on secondary chemical related treatments prior to dosage with virus (Hoover, 1998; Ali et al., 2002), and 3) after infection, when the possibility of self-medication is examined (Young et al., 1995; Lee et al., 2006; Singer et al., 2009). However, such bioassays do not allow for natural behaviour and feeding preferences that would come into play in the field (as has been studied for the gypsy moth, *L. dispar* [Barbosa & Krischik, 1987;

Dwyer, 1991; Osier et al., 2000], among other insects), which may moderate harmful effects from excessive PSC consumption.

Although the effects of PSCs against baculoviruses within a generation have been studied, how transgenerational effects of plant induction lead to increased viral resistance and upregulated immunity in offspring is a black box. Rotem et al. (2003) examined the potential for cross-generational effects from increasing allelochemical (glucosinolate) concentration in artificial diet for the Small White butterfly (*Pieris rapae*), but found no evidence for transgenerational effects from variable parental allelochemical consumption. However, allelochemical content of induced foliage is likely diverse and different in red alder (*A. rubra*), and numerous other changes could be taking place in foliage from damaged branches (Karban & Baldwin, 1997; Schaller, 2008). Additionally, *P. rapae* is a specialist on Brassicacae (of which many species contain glucosinolates), and is highly co-evolved to deal with the presence of such an allelochemical (Rotem et al., 2003). Generalists, such as the WTC, may be less adapted to high concentrations of specific allelochemicals (review in Heckel, 2014), meaning that transgenerational effects in response to allelochemicals may be more likely in a generalist herbivore. Insect herbivores have evolved multiple strategies to detoxify, excrete, and even utilize defensive compounds produced by plants, though these strategies often carry metabolic costs (see reviews in Nishida, 2002; Després, David & Gallet, 2007; Heckel, 2014). As a generalist herbivore, it is likely that the WTC must express a range of detoxification strategies (Krieger et al., 1971; Heckel, 2014), though little is known about secondary chemical detoxification in tent caterpillars (Fitzgerald, 1995; Hemming & Lindroth, 2000).

If we view the consumption of induced foliage more broadly as a poor diet, potentially involving metabolic costs to PSCs, the transgenerational effect of plant induction on disease resistance fits with the premise that parents may be able to prepare offspring for predicted environmental conditions based on those they experienced, i.e. adaptive transgenerational plasticity (Mousseau & Fox, 1998; Hunter, 2002; Räsänen & Kruuk, 2007). As an increased risk of disease may be linked with sub-optimal environments (Mitchell & Read, 2005; Ben-Ami et al., 2010; Boots & Roberts, 2012; Garbutt et al., 2013; Scholtz, Ebert, & Martin-Creuzburg, 2013), it may be expected that parents would invest more in offspring resistance to pathogens when fed a sub-optimal

diet consisting of induced foliage, as this may be correlated with high density populations and increased risk of infection.

Increased disease resistance seen in offspring of induced-foliage-fed insects was matched by upregulation of PO activity and encapsulation rate (the latter was only increased in offspring of insects fed induced foliage without bacteria – see below). Although I am unaware of any studies reporting differences in immunity from transgenerational plant induction, within generation studies have investigated this but have reported equivocal effects (Kapari et al., 2006; Haviola et al., 2007; Martemyanov et al., 2012; Campo et al., 2012). For example, Martemyanov et al. (2012b) found that both the encapsulation ability and lysozyme-like activity of the gypsy moth *L. dispar*, was upregulated by rapid same season induced resistance of it's host tree, the silver birch (*Betula pendula*), while another recent study on the same system found no evidence for an effect of RIR on larval encapsulation ability but a sex specific effect on haemocyte density (Martemyanov et al. 2013).

Patterns seen for PO and encapsulation in offspring of induced foliage fed insects may be related to their higher virus resistance. As PO is involved in the melanisation pathway, it may act at the midgut level, leading to the encapsulation of infected mid-gut tracheal cells (Washburn et al., 1996, 2000). Though PO may be correlated with virucidal activity against baculoviruses (Shelby & Popham, 2006; Wilson et al. 2001), this has not been a consistent pattern (Shikano et al., 2010; Saejeng et al. 2010, Myers et al., 2011; review in González-Santoyo & Córdoba-Aguilar, 2011). However, there is substantial evidence that PO is an important indicator of host condition (González-Santoyo & Córdoba-Aguilar, 2011). The melanisation response was lower in offspring of parents fed a combination of induced foliage and bacteria versus offspring of parents fed induced but naïve foliage, suggesting that the presence of bacteria in the parental diet somehow cancels out the heightened melanisation response otherwise seen with parents fed induced foliage. Although no previous studies have found evidence for transgenerational immune-related trade-offs, Cotter et al. (2004) found a genetic trade-off between antibacterial activity and haemocyte density (correlated with both PO and encapsulation ability) in the Egyptian cotton leafworm *Spodoptera littoralis*. It is possible that parental exposure to bacteria led to an upregulation in antibacterial

activity of offspring haemolymph at a cost to the immune pathway involving melanisation/encapsulation or other unknown or unmeasured virus resistance mechanisms. However, I found no evidence of differences in offspring haemocyte density or PO activity due to parental consumption of bacteria, which does not add credibility to this explanation.

As already mentioned, offspring of parents fed bacteria had slightly higher resistance to virus, indicating a generalized response to cross-generational immune challenge and running contrary to my original predictions. However, it is worth considering if this would truly be the expected response based on current knowledge. Innate, rather than adaptive or acquired immunity is considered the rule of thumb for invertebrates (Hoffman & Reichhart, 2002; Rowley & Powell, 2007), and the invertebrate immune system lacks antigen-specific lymphocytes and immune memory cells found in vertebrates (Arala-Chaves & Sequiera, 2000; Janeway & Medzhitov, 2002; Schmid-Hempel, 2005). What's more, it has been argued that invertebrate life cycles are usually too brief to warrant investment in protection against secondary pathogen exposure, as immune upregulation is costly (Little & Kraaijeveld, 2004). However, the invertebrate immune system may be able to act in an adaptive manner resembling that of vertebrates, as primary encounters with pathogens or immune insults have been shown to provide protection upon subsequent challenges (review in Rowely & Powell, 2007; Moret and Siva-Jothy, 2003; Korner & Schmid-Hempel, 2004; Mowlds et al., 2008; Sadd & Schmid-Hempel, 2006; Roth et al., 2009b; but see Vorberger et al., 2008; Linder & Promislow, 2009). This immune priming may come in the form of a generalized upregulation in immunocompetence in insects (Moret and Siva-Jothy, 2003; Korner & Schmid-Hempel, 2004; Mowlds et al., 2008). But there is also emerging evidence for specificity for insect immune priming, varying from fine-grained (i.e. specificity among strains of a particular species or related species) (Sadd & Schmid-Hempel, 2006; Roth et al., 2009b) to coarse-grained, (i.e. specificity between more general groups, e.g. fungi, Gram-positive and Gram-negative bacteria) (Hoffman, 2003).

The issue of invertebrate longevity in relation to the value of immune priming is transcended if such effects are considered across generations (Sadd et al., 2005). The phenomenon of TGIP is based on the idea that parental immune challenge acts as a

predictor for pathogen risk in offspring, and specificity involving costs to heterologous challenges in offspring have been documented (Little et al., 2003; Moret, 2006; Sadd & Schmid-Hempel, 2009). The finding that parental consumption of bacteria benefited offspring virus resistance was thus somewhat surprising, as I am unaware of studies that found generality for microorganism-based transgenerational effects. Yet, this result makes more sense if the presence of foliar bacteria in the parental diet is again thought of as a stressor implicated with a poor environment and increased pathogen risk (Mitchell & Read, 2005; Ben-Ami et al., 2010; Boots & Roberts, 2012; Garbutt et al., 2013; Scholtz et al., 2013).

Although parental consumption of bacteria led to offspring that were more virus resistant, the larvae that were infected died faster than those reared from parents raised on naïve foliage. This indicates that bacteria could have influenced the relationship between McplNPV virulence and transmission in offspring. Earlier death means that that virus can initiate secondary rounds of infection more rapidly (Woods & Elkinton, 1987); however, the difference in time to death was relatively small, (12 hours), and therefore may hold little biological significance in terms of infection dynamics. However, the biological significance of speed of kill rates is dependent upon specific natural conditions and competition with other pathogens and predators (Hodgson et al., 2001); host-pathogen models (Dwyer et al., 2000; Fuller, Elderd, & Dwyer, 2012) may be able to address the ecological relevance of such a difference.

It is curious that despite effects on offspring disease resistance and fitness, I found no measurable difference in offspring immune function from parental consumption of bacteria. Previous work by Freitak et al. (2009a,b) with *T. ni* indicated transgenerational effects on immune function, increasing offspring PO levels and expression of immune-related proteins. I was unable to obtain any results from the antibacterial assay, which could be due to 1) inadequate volume of haemolymph samples or excessive dilution of said samples or 2) the relatively long delay (~3 months) between sample collection/storage and eventual analysis. It is possible that antibacterial haemolymph activity would have be upregulated in offspring of parents fed foliar bacteria.

One may have expected changes in disease resistance and immunity in offspring to be linked with trade-offs in other life history characteristics, and this was the case. Female pupal mass, a strong predictor of Lepidopteran fecundity (Tammaru & Haukioja, 1996; Tammaru et al., 1996; Calvo & Molina, 2005), was lower in offspring of parents fed induced foliage or bacteria. The decrease in pupal weight and growth rate in female progeny of induced-foliage-fed insects is contrary to that of Rossiter (1991), who found that offspring of *L. dispar* mothers fed on red oak (*Q. rubra*) foliage with higher herbivore damage had decreased pupal weights and fecundity but produced offspring that were *heavier* as pupae. Apart from the differences between the two species, this difference is likely due to different methodologies; *L. dispar* larvae were reared on induced foliage for their whole larval period but were reared on artificial diet in the offspring generation. In addition, the *L. dispar* defoliation treatment was more severe (and allowed for feeding on damaged leaves). Thus there might a threshold between positive and negative effects – while my results were representative of a positive maternal effect (i.e. mothers with suboptimal growth led to offspring with suboptimal growth and mass), the effect described by Rossiter (1991) is classed as a negative maternal effect (i.e. lighter mothers produced heavier offspring). I argue that intense defoliation (among other changes) during peak populations of WTC would represent an environment undergoing a high level of flux, thus fitting the observation of positive maternal effects on offspring life history.

Decreased pupal weight of female offspring from parents fed bacteria agrees with a recent TGIP study in *T. ni* by Shikano et al. (in press), which found that parents challenged with a sublethal dose of *Bacillus thuringiensis* gave rise to offspring with lower pupal masses (reared on a standard diet). However, most other studies on TGIP in insects (Moret 2006; Freitak et al. 2009b; Trauer & Hilker, 2013; McNamara et al. 2014) find either no effect or a positive influence on offspring pupal mass/size, though some of these effects could be the result of selection (e.g. McNamara et al., 2014). As an example of a contrary effect, Trauer and Hilker (2013) reported that female offspring of tobacco hornworm *M. sexta* parents injected with peptidoglycan (PGN), a component of bacterial cell walls, had higher pupal masses vs. offspring of non-injected parents. However, this resulted in lower fecundity in offspring, and showed a cost to

transgenerational immune priming, possibly related to diversion of resources from egg production to synthesis of antimicrobial peptides.

Another interesting pattern in life history was that the initial (4th instar) mass of offspring was positively influenced by a parental diet of induced foliage or bacteria. Growth from hatch to the 4th instar was more rapid versus offspring of control or naïve foliage fed insects, but shifted to an opposite relationship with final (pupal) mass. This decrease in later growth could have been due to a shift to enhanced immune function, balanced by a cost to pupal mass and fecundity, as has been shown for the tobacco hornworm *M. sexta* (Trauer & Hilker, 2013). In other invertebrates, stressful parental environments can increase the growth of juvenile offspring with potential tradeoffs in later periods of life (Marshall, 2008; Frost et al., 2010) It is important, however, to point out that 4th instar mass was not a significant covariate in the virus assay; therefore, the enhanced virus resistance was likely correlated with costs to later development. Differences in developmental patterns and size may be relevant to the WTC/McplNPV relationship, as foraging patterns and food demands change over time. Effects on interfamily/overall movement in trees and on ground surfaces influences the spread of disease and contact with virus OBs (Beisner & Myers, 1999).

Although I was unable to evaluate the transgenerational effects of all three stressors in conjunction, I still expected that an interaction between induced foliage and bacteria consumed in the parental generation would negatively affect offspring virus resistance. For this to be the likely, I expected that a combination of these two stressors would have incurred serious costs on parental life history, preventing adequate provisioning of offspring in the face of both nutritional/physiological stress imposed by feeding on induced foliage and immune activation through consumption of foliar bacteria.

While the combined cost of induced foliage and bacteria to parental growth was greater than either stressor alone (an interactive, non-additive effect), effects on key parameters such as fecundity or survival were absent. This might indicate that parents were not forced to make trade-offs between tolerating nutritional stress and activating immunity (Siva-Jothy & Thompson, 2002; Myers et al., 2011; Triggs & Knell, 2012; Shikano et al., in press). Few studies have investigated how combined stressors act

across generations on disease susceptibility or immunocompetence of insects (Zanchi et al., 2012; Shikano et al., in press) or other invertebrates (Mitchell & Read, 2005; Coors & De Meester, 2008; Garbutt et al., 2013). There is no consensus based on these studies, as combined parental stressors have produced transgenerational effects with negative (Coors & De Meester, 2008), neutral (Zanchi et al., 2012; Shikano et al., in press) or positive (Mitchell & Read, 2005; Garbutt et al., 2013) effects on disease resistance and immune function. In the current study, the presence of combined parental stressors on offspring was usually additive (i.e. sum of the individual effects), suggesting no emergent synergism or antagonism between the two stressors (Sih et al., 1998). The outcome of multiple stressors on transgenerational disease resistance is likely dependent upon complex relationships between the intensity and nature of stressors as well as the adaptive potential of transgenerational effects. The current study echoes this complexity inherent in such interactions and furthers the importance of viewing stressors contextually, as opposed to static and easily predictable.

3) Correlations between egg size/quality and offspring fitness

Some, but not all, life history parameters measured in offspring supported the concept that smaller eggs produce less fit offspring. The larvae hatching from eggs laid by insects fed on induced foliage (which should have been smaller as was found in the subsample measured) grew more slowly during later development and produced smaller female (but not male) pupae in offspring. Smaller eggs generally produce less fit offspring across a wide range of taxa (Azevedo, French, & Partridge, 1997), including arthropods (see review in Fox & Czesak, 2000; Torres-Vila & Rodrigues-Molina, 2002; Benton et al., 2005; Fox, 1997; Gliwicz & Guisande 1992). Yet, in some cases egg size has had no discernable effects on offspring life history in Lepidoptera (Karlsson & Wiklund, 1984, 1985; Karl et al., 2007). Although costs in offspring of decreased pupal mass and growth rate were associated with smaller eggs, these eggs also produced the insects that were most resistant to virus. This is contrary to the findings of Gibbs et al. (2010), who found a positive correlation between egg size and offspring baculovirus (AcMNPV) resistance in the speckled wood butterfly *Pararge aegeria*. Smaller eggs could even be correlated with increased immune function in offspring. For example, Shoemaker and Adamo (2007) injected field crickets, *Gryllus texensis*, with LPS and

found that these immune challenged mothers laid smaller eggs. Sadd & Schmid-Hempel, (2007) reported that immune challenge in mothers can increase immune-related provisioning in eggs, carrying over to offspring with enhanced immune function. I speculate that smaller WTC eggs could possess a level of immune function equal to or greater than larger eggs laid by non-challenged females. Although the effect of consuming induced foliage undoubtedly differs from the stress of an immune challenge, it is likely that egg size is not a reliable metric for predicting immunocompetence and that egg quality is a better predictor of fitness (Fox & Czesak, 2000; McIntyre & Gooding, 2000; Giron & Casas, 2003).

Interestingly, eggs produced by mothers fed foliar bacteria with higher amounts of protein correlated with increases in the following traits of offspring: 1) baculovirus resistance, 2) initial (4^{th} instar) mass, 3) emergence success rate in virus-challenged survivors, 4) and increased overall survival to pupation. In insects, higher protein content in eggs may be positively correlated with offspring fitness, especially for early survival and dispersal (Diss et al., 1996; Rossiter et al., 1996; Chapman, 1998). Thus there is some evidence that egg provisioning, if not size, was linked to the disease resistance of the offspring, but more work would be required to determine what form this takes and how it links to disease resistance.

4) The potential roles of epigenetics or gene regulation

It is unlikely that the transgenerational effects seen in this study are due to a single mechanism; the effects on offspring are likely to be the product of an array of processes occurring throughout parental development, the egg stage, and phenotypic expression in offspring. Mothers may have differentially provisioned offspring based on environmental experience, but parental dietary factors could also influence offspring through epigenetic changes, resulting in differences relating to gene expression that are independent of resources.

There is evidence that exposure to non-pathogenic bacteria can influence gene expression both within and across generations. Within a generation, exposure of Lepidopteran larvae to non-pathogenic bacteria (*E. coli* and *M. luteus*) has been shown to increase the expression of immune genes, antimicrobial peptides, or pattern

recognition proteins (Aye et al., 2004; Freitak et al., 2007). Freitak et al. (2009a) also showed that parental consumption of *E. coli* and *M. luteus* in *T. ni*, resulted in differences in gene expression in eggs, as well as increased expression of immune-response-related genes, including hemolin, as well as storage-related proteins (e.g. vitellogenin) in offspring. Evidence for genomic imprinting by methylation has been found in Lepidoptera and other insects (Xiang et al., 2010; Glastad et al., 2011; Anaka et al., 2009; Elango et al., 2009). Triggs & Knell (2012b) speculated that the transgenerational results in their study were the result of epigenetic changes rather than differences in egg provisioning, based on transgenerational effects arising from both mothers and fathers, as well as the relative influence of parental diet on immune measures in offspring reared on optimal diet.

5) Implications of transgenerational effects on WTC population dynamics

Manipulative population-level studies on cyclic forest insects are difficult to undertake both logistically (scale and complexity) and temporally (owing to long periods between cycles), and parsing out large-scale mechanisms behind cycles is a challenging task. Small-scale experiments, such as the current study can provide information about the potential mechanisms behind large scale patterns observed in the field, especially when such data are integrated into theoretical population modeling (Barbosa et al., 2012).

Although this study focused largely on exploring how changes in individual quality due to differences in dietary factors could contribute to cross-generational effects on disease susceptibility, the specific goal of this research was to investigate how an environment changing with population increase alters individual larval condition potentially precipitating population declines in the WTC. Wellington (1957, 1960, 1964, 1965) was an early proponent of the idea that WTC population cycles are influenced by changes in larval quality with increasing population density and changing environmental factors, though most of the variation he proposed (involving weather, larval “activity”, and tent shape) is not supported by existing data (Myers, 2000). Despite this, the basic premise he put forth may still prove important for elucidating the cyclic behaviour of the WTC when applied transgenerationally.

Even though it was not possible to test the three-way interaction of dietary stressors on offspring, the observed transgenerational effects may provide clues to how these factors might act at the population level. In this experiment, transgenerational effects due to parental stress factors had mainly positive effects on disease resistance in offspring; this may seem counterintuitive when considering that these stressors are likely present in peak WTC populations. For example, on Galiano Island larval survival generally decreases before the population peak (Fig. 2.17, region 1) (Myers, 2000), suggesting density-related changes in environmental factors correlate with higher individual survival during the first half of population increase. Virus infection rates increase abruptly in most cases, and don't peak until larval survival reaches its nadir (Fig. 2.17, region 2) (Myers, 2000). If survival is amplified by the presence of either induced foliage and/or increased amounts of phylloplane bacteria, then the results of this study may be more relevant to the first 50% to 75% of the increase phase in population cycles, as opposed to representing the tipping point of populations.

Intriguingly, WTC population data indicate that fecundity increases up until two to four years before virus epizootics occur (generally *after* individual survival begins to decline; Fig. 2.17, region 3 (note that estimates of fecundity were based on counts of egg masses laid in the *prior* season) (Myers, 2000), which may indicate that fecundity is one of the last aspects of WTC life history to be negatively affected by the cumulative effect of intense stress factors in the environment. However, offspring of parents reared on a food-limited diet experienced no reduction in pupal mass, suggesting that multigenerational effects on body mass and fecundity from food limitation may not play a large role, although within generation effects likely lead to heavy selection pressures; if only *relatively* larger females survive food limitation and reproduce, this may confound any maternal effects from starvation. Additionally, offspring in field situations are likely to be exposed to defoliated conditions, induced plant defences, and virus contamination rather than control foliage as this experiment tested. In real world situations, these effects are likely tied together and can have negative effects on fitness across generations (Rothman, 1997).

If food limitation plays an integral part in WTC population dynamics, the results of this experiment (and others) suggest that moths that were food-limited as larvae have

reduced fecundity. Both within the parental generation and compared to progeny of ad libitum larvae, food-limited larvae succumbed more frequently to unidentified pathogens. Food limitation may be integral to the precipitous population declines seen in the WTC. Therefore, as a factor in WTC population dynamics, food limitation is likely to have the greatest impact late in population increases, in addition to other stressors that have already intensified with defoliation, such as reduced leaf quality and increased phyloplane microbiota.

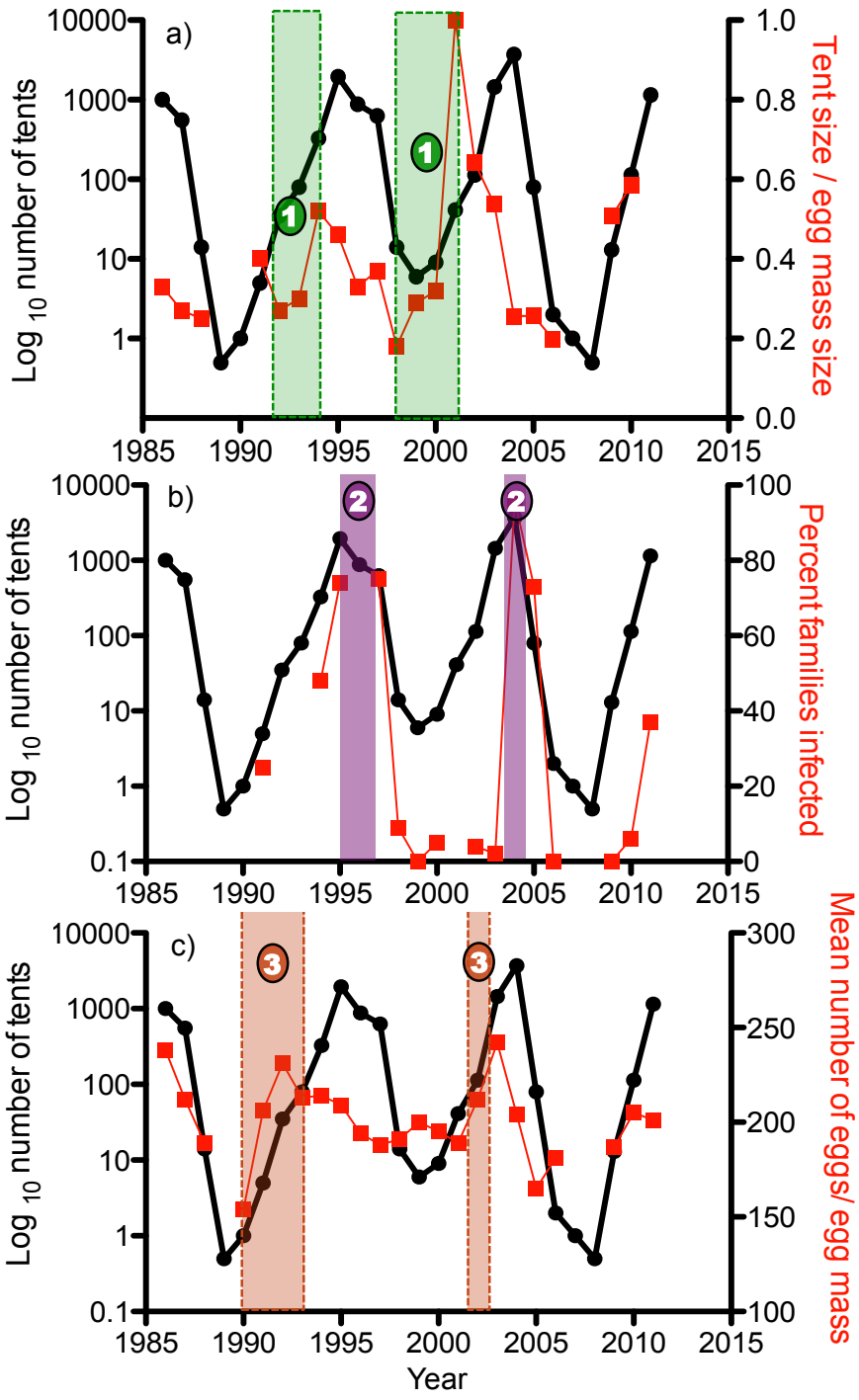


Figure 2.16. Population size of *M. c. pluviale* on Galiano Island, with y-axis plotted against year (x-axis) with 2° y-axes as: (a) survival, (b) infection, and (c) fecundity. Colors of shaded regions: (1) green = population growth with increasing individual quality, (2) purple = peak of virus infection/nadir of larval survival, and (3) orange = peak fecundity range. Figure modified from Myers & Cory (2013).

Future directions and conclusions

The mechanisms behind the transgenerational effects observed in this study are as yet unknown; it would be valuable to evaluate the potential for epigenetic changes due to transgenerational factors in the WTC, especially relating to the expression of immune-related genes and proteins. Future experiments could explore the relative importance of density-related environmental stressors in association with egg provisioning and epigenetics.

Carryover effects past the second generation, such as growth, age at maturity, and reproductive output, have been found in other invertebrates (Hafer et al., 2011; Benton et al., 2005), but no such effects are known that arise from plant induction of consumption of non-pathogenic bacteria. Along with the transgenerational effects observed in this study, the fact that pupal masses of female offspring (an indicator of fecundity) differed due to parental diets of induced foliage and foliar bacteria suggests that transgenerational effects may carry on into the 3rd generation and this warrants further exploration.

The transgenerational factors explored in this study indicate that plant induction and bacterial priming act on parents to produce progeny that are more resistant to disease. The implications for this research may extend to other cyclic forest Lepidoptera and other species. Further exploration of environmental based transgenerational factors can provide new insights into questions surrounding the role of the environmental variability on the host-pathogen relationship.

2.C. References

Abbott, K. C., & Dwyer, G. (2007). Food limitation and insect outbreaks: complex dynamics in plant-herbivore models. *The Journal of Animal Ecology*, *76*(5), 1004–14.

Adams, A. B. (1989). *The relationship between the leaf nutritional quality of Alnus rubra Bong., and the relative fitness of Malacomsoma californicum pluviale (Dyar).* University of Washington, Seattle.

Alalouni, U., Schädler, M., & Brandl, R. (2013). Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. *Journal of Applied Entomology*, *137*(10), 721–738.

Ali, M. I., Young, S. Y., Felton, G. W., & McNew, R. W. (2002). Influence of the host plant on occluded virus production and lethal infectivity of a baculovirus. *Journal of Invertebrate Pathology*, *81*(3), 158–65.

Anaka, M., Lynn, A., McGinn, P., & Lloyd, V. K. (2009). Genomic imprinting in *Drosophila* has properties of both mammalian and insect imprinting. *Development Genes and Evolution*, *219*(2), 59–66.

Anderson, R. M., & May, R. M. (1978). Regulation and stability of host-parasite interactions: I. Regulatory processes. *Journal of Animal Ecology*, *47*(1), 219–247.

Anderson, R. M., & May, R. M. (1981). The Population Dynamics of Microparasites and Their Invertebrate Hosts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *291*(1054), 451–524.

Andrews, J. H., Kenerley, C. M., & Nordheim, E. V. (1980). Positional variation in phylloplane microbial populations within an apple tree canopy. *Microbial Ecology*, *6*(1), 71–84.

Arala-Chaves, M., & Sequeira, T. (2000). Is there any kind of adaptive immunity in invertebrates? *Aquaculture*, *191*(1-3), 247–258.

Aye, T. T., Lee, K.-Y., & Kwon, Y. J. (2004). Cloning of immune protein hemolin cDNA from the indian meal moth, *Plodia interpunctella*, and its High Induction During Development and by Bacterial Challenge. *Entomological Research*, *34*(4), 269–275.

Azevedo, R. B. R., French, V., & Partridge, L. (1997). Life - History Consequences of Egg Size in *Drosophila melanogaster*. *The American Naturalist*, *150*(2), 250–282.

Barbehenn, R. V, Jaros, A., Lee, G., Mozola, C., Weir, Q., & Salminen, J.-P. (2009). Tree resistance to *Lymantria dispar* caterpillars: importance and limitations of foliar tannin composition. *Oecologia*, *159*(4), 777–88.

Barbosa, P., & Krischik, V. A. (1987). Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria dispar*. *The American Naturalist*, *130*(1), 53–69.

Barbosa, P., Letourneau, D., & Agrawal, A. (Eds.). (2012). *Insect outbreaks revisited*. John Wiley & Sons.

Barnes, A. I., & Siva-Jothy, M. T. (2000). Density-dependent prophylaxis in the mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. *Proceedings. Biological Sciences / The Royal Society*, *267*(1439), 177–82.

Bauerfeind, S. S., & Fischer, K. (2008). Maternal body size as a morphological constraint on egg size and fecundity in butterflies. *Basic and Applied Ecology*, *9*(4), 443–451.

Beckage, N. E. (Ed.). (2011). *Insect immunology*. Academic Press.

Beckerman, A., Benton, T. G., Ranta, E., Kaitala, V., & Lundberg, P. (2002). Population dynamic consequences of delayed life-history effects. *Trends in Ecology & Evolution*, *17*(6), 263–269.

Beisner, B. E., & Myers, J. H. (1999). Population density and transmission of virus in experimental populations of the western tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, *28*(6), 1107–1113.

Beldomenico, P. M., & Begon, M. (2009). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology & Evolution*, *25*(1), 21–7.

Beldomenico, P. M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., & Begon, M. (2008). Poor condition and infection: a vicious circle in natural populations. *Proceedings. Biological Sciences / The Royal Society*, *275*(1644), 1753–9.

Ben-Ami, F., Ebert, D., & Regoes, R. R. (2010). Pathogen dose infectivity curves as a method to analyze the distribution of host susceptibility: a quantitative assessment of maternal effects after food stress and pathogen exposure. *The American Naturalist*, *175*(1), 106–15.

Benton, T. G., Plaistow, S. J., Beckerman, a P., Lapsley, C. T., & Littlejohns, S. (2005). Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society of B: Biological Sciences*, *272*(1570), 1351–6.

Benton, T. G., Ranta, E., Kaitala, V., & Beckerman, A. P. (2001). Maternal effects and the stability of population dynamics in noisy environments. *Journal of Animal Ecology*, *70*(4), 590–599.

Bergelson, J. (1994). The effects of genotype and the environment on costs of resistance in lettuce. *The American Naturalist*, *143*(2), 349–359.

Berretta, M. F., Ferrelli, M. L., Salvador, R., & Sciocco, A. (2013). Baculovirus Gene Expression. In *Current Issues in Molecular Virology- Viral Genetics and Biotechnological Applications* (pp. 57–78).

Berryman, A. A. (1987). Equilibrium or nonequilibrium: is that the question? *Bulletin of the Ecological Society of America*, *68*(4), 500–502.

Berryman, A. A. (1996). What causes population cycles of forest Lepidoptera? *Trends in Ecology & Evolution*, *11*(1), 28–32.

Berryman, A. A. (2002). Population cycles: causes and analysis. In A. A. Berryman (Ed.), *Population Cycles: The Case for Trophic Interactions* (pp. 3–28). Oxford Univ. Press.

Bettencourt, R., Assefaw-Redda, Y., & Faye, I. (2000). The insect immune protein hemolin is expressed during oogenesis and embryogenesis. *Mechanisms of Development*, *95*(1-2), 301–4.

Bettencourt, R., Lanz-Mendoza, H., Lindquist, K. R., & Faye, I. (1997). Cell adhesion properties of hemolin, an insect immune protein in the Ig superfamily. *European Journal of Biochemistry / FEBS*, *250*(3), 630–7.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, *144*(3), 353–61.

Boots, M., & Begon, M. (1994). Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth, *Plodia interpunctella*. *Ecological Entomology*, *19*, 319–326.

Boots, M., & Roberts, K. E. (2012). Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proceedings of the Royal Society B: Biological Sciences*, (July).

Bradford, G. E., & Famula, T. R. (1984). Evidence for a major gene for rapid postweaning growth in mice. *Genetical Research*, *44*(3), 293–308.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248–254.

Brett JR (1958) Implications and assessments of environmental stress. In: Larkin PA (eds) The investigations of fish power problems, HH MacMillian lecture series in fisheries. University of British Columbia, Vancouver, pp 69–83.

Broderick, N. A, Raffa, K. F., & Handelsman, J. (2006). Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(41), 15196–9.

Broderick, N. a, Robinson, C. J., McMahon, M. D., Holt, J., Handelsman, J., & Raffa, K. F. (2009). Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biology*, *7*, 11.

Broderick, N. A., Raffa, K. F., Goodman, R. M., & Handelsman, J. (2004). Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology*, *70*(1), 293–300.

Brown, D. G., & Weis, A. E. (1995). Direct and indirect effects of prior grazing of goldenrod upon the performance of a leaf beetle. *Ecology*, *76*(2), 426–436.

Burden, J. P., Griffiths, C. M., Cory, J. S., Smith, P., & Sait, S. M. (2002). Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Molecular Ecology*, *11*(3), 547–55.

Burden, J. P., Nixon, C. P., Hodgkinson, A. E., Possee, R. D., Sait, S. M., King, L. a., & Hails, R. S. (2003). Covert infections as a mechanism for long-term persistence of baculoviruses. *Ecology Letters*, *6*(6), 524–531.

Burden, J. P., Possee, R. D., Sait, S. M., King, L. a, & Hails, R. S. (2006). Phenotypic and genotypic characterisation of persistent baculovirus infections in populations of the cabbage moth (*Mamestra brassicae*) within the British Isles. *Archives of Virology*, *151*(4), 635–49.

Burnside, R., Schultz, M., Lisuzzo, N., & Kruse, J. (2013). Assessing mortality and regeneration of Larch (*Larix laricina*) after a 1999-2004 landscape level outbreak of the larch sawfly (*Pristophora erichsonii*) in Alaska (Project WC-EM-08-03). In K. M. Potter & B. L. Conkling (Eds.), *Forest Health Monitoring: National Status, Trends, and Analysis* (pp. 143–150). United States Department of Agriculture - Forest Service.

Calvo, D., & Molina, J. M. (2005). Fecundity–body size relationship and other reproductive aspects of *Streblote panda* (Lepidoptera: Lasiocampidae). *Annals of the Entomological Society of America*, *98*(2), 191–196.

Campero, M., De Block, M., Ollevier, F., & Stoks, R. (2008). Correcting the short-term effect of food deprivation in a damselfly: mechanisms and costs. *The Journal of Animal Ecology*, *77*(1), 66–73.

Carisey, N., & Bauce, E. (1997). Impact of balsam fir flowering on pollen and foliage biochemistry in relation to spruce budworm growth, development and food utilization. *Entomologia Experimentalis et Applicata*, *85*(1), 17–31.

Carisey, N., & Bauce, E. (2002). Does nutrition-related stress carry over to spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) progeny? *Bulletin of Entomological Research*, *92*(2), 101–8.

Chapman, R. F. (1998). *The insects: structure and function.* (4th editio.). Cambridge university press.

Cheng, Y.-S., Zheng, Y., & VanderGheynst, J. S. (2011). Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. *Lipids*, *46*(1), 95–103.

Clem, R. (2007). Baculoviruses and apoptosis: a diversity of genes and responses. *Current Drug Targets*, *8*(10), 1069–1074.

Colasurdo, N., Gélinas, Y., & Despland, E. (2009). Larval nutrition affects life history traits in a capital breeding moth. *The Journal of Experimental Biology*, *212*, 1794–800.

Cook, S. P., Webb, R. E., Podgwaite, J. D., & Reardon, R. C. (2003). Increased mortality of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) exposed to gypsy moth nuclear polyhedrosis virus in combination with the phenolic gycoside salicin. *Journal of Economic Entomology*, *96*(6), 1662–1667.

Cooper, D., Cory, J. S., & Myers, J. H. (2003). Hierarchical spatial structure of genetically variable nucleopolyhedroviruses infecting cyclic populations of western tent caterpillars. *Molecular Ecology*, *12*(4), 881–90.

Cooper, D., Cory, J. S., Theilmann, D. A., & Myers, J. H. (2003). Nucleopolyhedroviruses of forest and western tent caterpillars : cross-infectivity and evidence for activation of latent virus in high-density field populations. *Ecological Entomology*, *28*, 41–50.

Cooper, E. L. (2006). Comparative immunology. *Integrative Zoology*, *1*(1), 32–43.

Coors, A., & De Meester, L. (2008). Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in Daphnia magna. *Journal of Applied Ecology*, *45*, 1820–1828.

Cory, J. S., & Hoover, K. (2006). Plant-mediated effects in insect-pathogen interactions. *Trends in Ecology & Evolution*, *21*(5), 278–86.

Cory, J. S., & Myers, J. H. (2003). The Ecology and Evolution of Insect Baculoviruses. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 239–272.

Cory, J. S., & Myers, J. H. (2004). Adaptation in an insect host-plant pathogen interaction. *Ecology Letters*, *7*(8), 632–639.

Cory, J. S., & Myers, J. H. (2009). Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. *The Journal of Animal Ecology*, *78*(3), 646–55.

Cotter, S. C., Hails, R. S., Cory, J. S., & Wilson, K. (2004). Density-dependent prophylaxis and condition-dependent immune function in Lepidopteran larvae: a multivariate approach. *Journal of Animal Ecology*, *73*, 283–293.

Das, T. M. (1968). Physiological changes with leaf senescence: kinins on cell ageing and organ senescence. In *Proceedings of the International Symposium for Plant Growth Substances* (pp. 91–102).

Daxinger, L., & Whitelaw, E. (2012). Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Reviews. Genetics*, *13*(3), 153–62.

De Roode, J. C., Rarick, R. M., Mongue, A. J., Gerardo, N. M., & Hunter, M. D. (2011). Aphids indirectly increase virulence and transmission potential of a monarch butterfly parasite by reducing defensive chemistry of a shared food plant. *Ecology Letters*, *14*(5), 453–61.

De Vries, E. J., Jacobs, G., & Breeuwer, J. a. (2001). Growth and transmission of gut bacteria in the Western flower thrips, *Frankliniella occidentalis*. *Journal of Invertebrate Pathology*, *77*(2), 129–37.

Del Campo, M. L., Halitschke, R., Short, S. M., Lazzaro, B. P., & Kessler, A. (2013). Dietary plant phenolic improves survival of bacterial infection in *Manduca sexta* caterpillars. *Entomologia Experimentalis et Applicata*, *146*(3), 321–331.

Després, L., David, J.-P., & Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution*, *22*(6), 298–307.

DeWitt, T. J., & Scheiner, S. M. (Eds.). (2004). *Phenotypic plasticity: functional and conceptual approaches* (pp. 98–111). Oxford: Oxford University Press.

Dillon, R. J., & Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology*, *49*(98), 71–92.

Dillon, R. J., Webster, G., Weightman, A. J., & Keith Charnley, a. (2010). Diversity of gut microbiota increases with aging and starvation in the desert locust. *Antonie van Leeuwenhoek*, *97*(1), 69–77.

Diss, A. L., Kunkel, J. G., Montgomery, M. E., & Leonard, D. E. (1996). Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. *Oecologia*, *106*(4), 470–477.

Donegan, K., & Lighthart, B. (1989). Effect of several stress factors on the susceptibility of the predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen *Beauveria bassiana. Journal of Invertebrate Pathology*, *54*(1), 79–84.

Drew, R. A. I., Courtice, A. C., & Teakle, D. S. (1983). Bacteria as a natural source of food for adult fruit flies (Diptera : Tephritidae). *Oecologia*, *60*, 279–284.

Dwyer, G. (1991). The roles of density, stage ,and patchiness in the transmission of an insect virus. *Ecology*, *72*(2), 559–574.

Dwyer, G., Dushoff, J., Elkinton, J., & Levin, S. (2000). Pathogen-Driven Outbreaks in Forest Defoliators Revisited: Building Models from Experimental Data. *The American Naturalist*, *156*(2), 105–120.

Dwyer, G., & Elkinton, J. S. (1993). Using simple models to predict virus epizootics in gypsy moth populations. *Journal of Animal Ecology*, *62*(1), 1–11.

Elango, N., Hunt, B. G., Goodisman, M. a D., & Yi, S. V. (2009). DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(27), 11206–11.

Elderd, B. D., Rehill, B. J., Haynes, K. J., & Dwyer, G. (2013). Induced plant defenses, host-pathogen interactions, and forest insect outbreaks. Proceedings of the National Academy of Sciences of the United States of America, 110(37), 14978–14983.

Eleftherianos, I., Marokhazi, J., Millichap, P. J., Hodgkinson, A. J., Sriboonlert, A., ffrench-Constant, R. H., & Reynolds, S. E. (2006). Prior infection of *Manduca sexta* with non-pathogenic Escherichia coli elicits immunity to pathogenic Photorhabdus luminescens: roles of immune-related proteins shown by RNA interference. *Insect Biochemistry and Molecular Biology*, *36*(6), 517–25.

Elliot, N., Kieckhefer, R., & Kauffman, W. (1996). Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia*, *105*, 537–544.

Elton, B. Y. C., & Nicholson, M. (1942). The ten-year cycle in numbers of the lynx in Canada. *Journal of Animal Ecology*, *11*(2), 215–244.

Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - diversity in structure and function. *FEMS Microbiology Reviews*, *37*(5), 699–735.

Erban, T., & Hubert, J. (2008). Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. *Experimental & Applied Acarology*, *44*(3), 199–212.

Ericsson, J. D., Janmaat, A. F., Lowenberger, C., & Myers, J. H. (2009). Is decreased generalized immunity a cost of Bt resistance in cabbage loopers *Trichoplusia ni*? *Journal of Invertebrate Pathology*, *100*(2), 61–7.

Faye, I., & Kanost, M. (1998). Function and regulation of hemolin. In P. T. Brey & D. Hultmark (Eds.), *Molecular mechanisms of immune responses in insects* (pp. 173–188). London: Chapman & Hall.

Feder, D., Mello, C. B., Garcia, E. S., & Azambuja, P. (1997). Immune responses in *Rhodnius prolixus*: influence of nutrition and ecdysone. *Journal of Insect Physiology*, *43*(6), 513–519.

Felton, G. W., & Duffey, S. S. (1990). Inactivation of baculovirus by quinones formed in insect-damaged plant tissues. *Journal of Chemical Ecology*, *16*(4), 1221–1236.

Ferrandon, D., Imler, J. L., Hetru, C., & Hoffmann, J. A. (2007). The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. *Nature Reviews. Immunology*, *7*(11), 862–74.

Ferro, D. N. (1985). Pest status and control strategies of the Colorado potato beetle. *Research Bulletin - Massachusetts Agricultural Experiment Station (USA)*.

Fischer, K., Brakefield, P. M., & Zwaan, B. J. (2003). Plasticity in butterfly egg size: why larger offspring at lower temperatures? *Ecology*, *84*(12), 3138–3147.

Fitzgerald, T. D. (1995). *The Tent Caterpillars*. New York: Cornell University Press.

Fitzpatrick, S. M., & Troubridge, J. T. (1993). Fecundity, number of diapause eggs, and egg size of successive generations of the blackheaded fireworm (Lepidoptera: Tortricidae) on cranberries. *Environmental Entomology*, *22*(4), 818–823.

Forbey, J., & Hunter, M. D. (2012). The ecology of plant secondary metabolites. In G. R. Iason, M. Dicke, & S. E. Hartley (Eds.), *The Herbivore's Prescription: A Pharm-Ecological Perspective on Host Plant Use by Vertebrate and Invertebrate Herbivores.* (p. 78). New York: Cambridge University Press.

Fox, C. W. (1997). Egg-size manipulations in the seed beetle *Stator limbatus:* consequences for progeny growth. *Canadian Journal of Zoology*, *75*, 1465–1473.

Fox, C. W., & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, *45*, 341–69.

Fox, C. W., Czesak, M. E., & Savalli, U. D. O. M. (1999). Environmentally based maternal effects on development time in the seed beetle *Stator pruininus* (Coleoptera : Bruchidae): Consequences of Larval Density. *Population Ecology*, *28*(2), 217–223.

Frago, E., & Bauce, E. (2014). Life-History Consequences of Chronic Nutritional Stress in an Outbreaking Insect Defoliator. *PloS One*, *9*(2), e88039.

Francis, D. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*, *286*(5442), 1155–1158.

Freitak, D., Heckel, D. G., & Vogel, H. (2009a). Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (*Trichoplusia ni*). *Frontiers in Zoology*, *6*, 7.

Freitak, D., Heckel, D. G., & Vogel, H. (2009b). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings. Biological Sciences / The Royal Society*, *276*(1667), 2617–24.

Freitak, D., Ots, I., Vanatoa, A., & Hõrak, P. (2003). Immune response is energetically costly in white cabbage butterfly pupae. *Proceedings. Biological Sciences / The Royal Society*, *270 Suppl* , S220–2.

Freitak, D., Wheat, C. W., Heckel, D. G., & Vogel, H. (2007). Immune system responses and fitness costs associated with consumption of bacteria in larvae of *Trichoplusia ni*. *BMC Biology*, *5*, 56.

Frost, P. C., Ebert, D., Larson, J. H., Marcus, M. a, Wagner, N. D., & Zalewski, A. (2010). Transgenerational effects of poor elemental food quality on *Daphnia magna*. *Oecologia*, *162*(4), 865–72.

Fuller, E., Elderd, B. D., & Dwyer, G. (2012). Pathogen persistence in the environment and insect-baculovirus interactions: disease-density thresholds, epidemic burnout and insect outbreaks. *American Naturalist*, *179*(3), 1–30.

Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International journal of molecular sciences, 14*, 10242–97).

Futuyma, D. J., Herrmann, C., Milstein, S., & Keese, M. C. (1993). Apparent transgenerational effects of host plant in the leaf beetle *Ophraella notulata* (Coleoptera: Chrysomelidae). *Oecologia*, *96*, 365–372.

Fuxa, J. R., Sun, J. Z., Weidner, E. H., & LaMotte, L. R. (1999). Stressors and rearing diseases of *Trichoplusia ni*: evidence of vertical transmission of NPV and CPV. *Journal of Invertebrate Pathology*, *74*(2), 149–55.

Garbutt, J. S., Scholefield, J. a., Vale, P. F., & Little, T. J. (2013). Elevated maternal temperature enhances offspring disease resistance in *Daphnia magna*. *Functional Ecology*, *28*(2), 424–431.

García-Lara, J., Needham, A. J., & Foster, S. J. (2005). Invertebrates as animal models for *Staphylococcus aureus pathogenesis*: a window into host-pathogen interaction. *FEMS Immunology and Medical Microbiology*, *43*(3), 311–23.

Gavery, M. R., & Roberts, S. B. (2010). DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (Crassostrea gigas). *BMC Genomics*, *11*, 483.

Geister, T. L., Lorenz, M. W., Hoffmann, K. H., & Fischer, K. (2008). Adult nutrition and butterfly fitness: effects of diet quality on reproductive output, egg composition, and egg hatching success. *Frontiers in Zoology*, *5*, 10.

Gibbs, M., Breuker, C. J., Hesketh, H., Hails, R. S., & Van Dyck, H. (2010). Maternal effects, flight versus fecundity trade-offs, and offspring immune defence in the speckled wood butterfly, Pararge aegeria. *BMC Evolutionary Biology*, *10*, 345.

Gillespie, J. P., Kanost, M. R., & Trenczek, T. (1997). Biological mediators of insect immunity. *Annual Review of Entomology*, *42*, 611–643.

Ginzburg, L. R., & Taneyhill, D. E. (1994). Population cycles of forest lepidoptera: a maternal effect hypothesis. *The Journal of Animal Ecology*, *63*(1), 79.

Giron, D., & Casas, J. (2003). Mothers reduce egg provisioning with age. *Ecology Letters*, *6*, 273–277.

Glastad, K. M., Hunt, B. G., Yi, S. V, & Goodisman, M. D. (2011). DNA methylation in insects: on the brink of the epigenomic era. *Insect Molecular Biology*, *20*(5), 553–65.

Gliwicz, Z. M., & Guisande, C. (1992). Family Planning in Daphnia : Resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia*, *91*(4), 463–467.

González-Santoyo, I., & Córdoba-Aguilar, A. (2011). Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata*, *142*(1), 1–16.

Gorman, M. J., Kankanala, P., & Kanost, M. R. (2004). Bacterial challenge stimulates innate immune responses in extra-embryonic tissues of tobacco hornworm eggs. *Insect Molecular Biology*, *13*(1), 19–24.

Grimbacher, P., Matsuki, M., Collett, N., Elek, J., & Wardlaw, T. (2011). *Are insect herbivores in Eucalyptus globulus/nitens plantations a worsening problem? A multi-region spatio-temporal review of southern Australia*. Technical Report No TR216. Cooperative Research Centre for Forestry, Australia.

Grindstaff, J. L. (2008). Maternal antibodies reduce costs of an immune response during development. *The Journal of Experimental Biology*, *211*(Pt 5), 654–60.

Grindstaff, J. L., Brodie, E. D., & Ketterson, E. D. (2003). Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings. Biological Sciences / The Royal Society*, *270*(1531), 2309–19.

Grindstaff, J. L., Demas, G. E., & Ketterson, E. D. (2005). Diet quality affects egg size and number but does not reduce maternal antibody transmission in Japanese quail Coturnix japonica. *Journal of Animal Ecology*, *74*(6), 1051–1058.

Gwynne, D. T. (1988). Courtship feeding and the fitness of female katydids (Orthoptera: Tettigoniidae). *Evolution*, *42*(3), 545–55.

Hafer, N., Ebil, S., Uller, T., & Pike, N. (2011). Transgenerational effects of food availability on age at maturity and reproductive output in an asexual collembolan species. *Biology Letters*, *7*(5), 755–8.

Hajek, A. E., & Leger, R. J. (1994). Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, *39*(1), 293–322.

Hajek, A. E., McManus, M. L., & Delalibera, I. (2007). A review of introductions of pathogens and nematodes for classical biological control of insects and mites. *Biological Control*, *41*(1), 1–13.

Hamilton, K. ., Nisbet, a. ., Lehane, M. ., Taylor, M. ., & Billingsley, P. . (2003). A physiological and biochemical model for digestion in the ectoparasitic mite, Psoroptes ovis (Acari: Psoroptidae). *International Journal for Parasitology*, *33*(8), 773–785.

Hanski, I., Henttonen, H., Korpimaki, E., Oksanen, L., & Turchin, P. (2001). Small-rodent dynamics and predation. *Ecology*, *82*(6), 1505–1520.

Harvey, G. T. (1985). Egg weight as a factor in the overwintering survival of spruce budworm (Lepidoptera: Tortricidae) larvae. *The Canadian Entomologist*, *117*(12), 1451–1461.

Haukioja, E. (2005). Plant defenses and population fluctuations of forest defoliators : mechanism-based scenarios. *Annual Zoology Fennici*, *42*, 313–325.

Haukioja, E., & Hanhimäki, S. (1985). Rapid wound-induced resistance in white birch (*Betula pubescens*) foliage to the geometrid *Epirrita autumnata*: a comparison of trees and moths within and outside the outbreak range of the moth. *Oecologia*, *65*(2), 223–228.

Haukioja, E., Niemel, P., & Siren, S. (1985). Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens ssp tortuosa*. *Oecologia*, *65*, 214–222.

Haukioja, E., & Niemela, P. (1979). Birch leaves as a resource for herbivores: seasonal occurence of increased resistance in foliage after mechanical damage of adjacent leaves. *Oecologia*, *39*, 151–159.

Haukioja, E., Ossipov, V., & Lempa, K. (2002). Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata*, *104*(1), 125–136.

Hauton, C., & Smith, V. J. (2007). Adaptive immunity in invertebrates: a straw house without a mechanistic foundation. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *29*(11), 1138–46.

Havill, N. P., & Raffa, K. F. (1999). Effects of elicitation treatment and genotypic variation on induced resistance in Populus : impacts on gypsy moth (Lepidoptera : Lymantriidae) development and feeding behavior. *Oecologia*, *120*, 295–303.

Haviola, S., Kapari, L., Ossipov, V., Rantala, M. J., Ruuhola, T., & Haukioja, E. (2007). Foliar phenolics are differently associated with *Epirrita autumnata* growth and immunocompetence. *Journal of Chemical Ecology*, *33*(5), 1013–23.

Hawkins, B. A. H., Cornell, H. V. C., & Hochberg, M. E. (1997). Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology*, *78*(7), 2145–2152.

Hawley, D. M., & Altizer, S. M. (2011). Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology*, *25*(1), 48–60.

Heckel, D. G. (2014). Insect detoxification and sequestration strategies. *Insect-Plant Interactions*, *47*, 77–114.

Hemming, J. D. C., & Lindroth, R. L. (2000). Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. *Environmental Entomology*, *29*(6), 1108–1115.

Hodgson, D. J., Vanbergen, A. J., Watt, A. D., Hails, R. S., & Cory, J. S. (2001). Phenotypic variation between naturally co-existing genotypes of a Lepidopteran baculovirus, 687–701.

Hoffmann, J. A. (2003). The immune response of *Drosophila*. *Nature*, *426*(6962), 33–8.

Hoffmann, J. A, & Reichhart, J.-M. (2002). Drosophila innate immunity: an evolutionary perspective. *Nature Immunology*, *3*(2), 121–6.

Hoover, K., Alaniz, S. A., Yee, J. L., Rocke, D. M., Hammock, B. D., & Duffey, S. S. (1998). Dietary protein and chlorogenic acid effect on baculoviral disease of noctuid (Lepidoptera: Noctuidae) larvae. *Environmental Entomology*, *27*(5), 1264–1272.

Hoover, K., Washburn, J., & Volkman, L. (2000). Midgut-based resistance of *Heliothis virescens* to baculovirus infection mediated by phytochemicals in cotton. *Journal of Insect Physiology*, *46*(6), 999–1007.

Howe, G. A., & Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology*, *59*, 41–66.

Hoyle, R. B., & Ezard, T. H. G. (2012). The benefits of maternal effects in novel and in stable environments. *Journal of the Royal Society, Interface / the Royal Society*, *9*(75), 2403–13.

Hudson, P. J., Rizzoli, A. P., Grenfell, B. T., Heesterbeek, J. A. P., & Dobson, A. P. (2002). *Ecology of wildlife diseases* (Vol. 1981, pp. 1–5). Oxford University Press.

Hugar, P., Kulkarni, K. A., & Lingappa, S. (1994). Studies on the transmission of nuclear polyhedrosis virus of sorghum armyworm, *Mythimna separata* (Walker) to its progenies. *Karnataka Journal of Agricultural Sciences*, *7*(4), 417–422.

Huitu, O., Jokinen, I., Korpimäki, E., Koskela, E., & Mappes, T. (2007). Phase dependence in winter physiological condition of cyclic voles. *Oikos*, *116*(4), 565–577.

Hunt, J., & Charnley, A. K. (1981). Abundance and distribution of the gut flora of the desert locust, *Schistocerca gregaria*. *Journal of Invertebrate Pathology*, *38*(3), 378–385.

Hunter, M. D. (2002). Maternal effects and the population dynamics of insects on plants. *Agricultural and Forest Entomology*, *4*(1), 1–9.

Hunter, M. D., & Schultz, J. C. (1993). Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia*, *94*, 195–203.

Il'inykh, A. V, & Ul'yanova, E. G. (2005). Latency of baculoviruses. *Biology Bulletin*, *32*(5), 496–502.

Inchausti, P., & Ginzburg, L. R. (2009). Maternal effects mechanism of population cycling: a formidable competitor to the traditional predator-prey view. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1520), 1117–24.

Indiragandhi, P., Anandham, R., & Sa, T. M. (2011). Functional significance of insect gut bacteria and their role in host insect processes, development, and crop production. In D. K. Maheshwari (Ed.), *Bacteria in Agrobiology: Plant Growth Responses*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Jakubowska, A. K., Vogel, H., & Herrero, S. (2013). Increase in gut microbiota after immune suppression in baculovirus-infected larvae. *PLoS Pathogens*, *9*(5), e1003379.

Janeway, C. A, & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, *20*(2), 197–216.

Janmaat, A. F., & Myers, J. H. (2005). The cost of resistance to *Bacillus thuringiensis* varies with the host plant of Trichoplusia ni. *Proceedings. Biological Sciences / The Royal Society*, *272*(1567), 1031–8.

Jiang, H., Vilcinskas, A., & Kanost, M. R. (2010). Chapter 10 Immunity in Lepidopteran insects. In K. Söderhäll (Ed.), *Invertebrate Immunity* (pp. 181–204). Landes Bioscience and Springer+Business Media.

Kaitaniemi, P., Ruohomaki, K., Tammaru, T., & Haukioja, E. (1999). Induced resistance of host tree foliage during and after a natural insect outbreak. *Journal of Animal Ecology*, *68*(2), 382–389.

Kapari, L., Haukioja, E., Rantala, M. J., & Ruuhola, T. (2006). Defoliating insect immune defense interacts with induced plant defense during a population outbreak. *Ecology*, *87*(2), 291–6.

Karamanoli, K., Menkissoglu-Spiroudi, U., Bosabalidis, A. M., Vokou, D., & Constantinidou, H.-I. a. (2005). Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. *Chemoecology*, *15*(2), 59–67.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, *25*(2), 339–347.

Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory*. University of Chicago Press.

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, *20*, 331–348.

Karl, I., Lorenz, M. W., & Fischer, K. (2007). Energetics of reproduction : consequences of divergent selection on egg size , food limitation , and female age for egg composition and reproductive effort in a butterfly. *Biological Journal of the Linnean Society*, *91*, 403–418.

Karlsson, B., & Wiklund, C. (1984). Egg weight variation and lack of correlation between egg weight and offspring in the wall brown fitness butterfly *Lasiommata megera*. *Oikos*, *43*(3), 376–385.

Karlsson, B., & Wiklund, C. (1985). Egg weight variation in relation to egg mortality and starvation endurance of newly hatched larvae in some satyrid butterflies. *Ecological Entomology*, *10*, 205–211.

Kaya, H. K., & Koppenhöfer, A. M. (1996). Effects of microbial and other antagonistic organisms and competition on entomopathogenic nematodes. *Biocontrol Science and Technology*, *6*(3), 357–372.

Keddie, B. a, Aponte, G. W., & Volkman, L. E. (1989). The pathway of infection of *Autographa californica* nuclear polyhedrosis virus in an insect host. *Science (New York, N.Y.)*, *243*(4899), 1728–30.

Kessler, a, & Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science (New York, N.Y.)*, *291*(5511), 2141–4.

Kessler, A., Poveda, K., & Poelman, E. H. (2012). Plant-induced responses and herbivore population dynamics. In P. Barbosa, D. K. Letourneau, & A. A. Agrawal (Eds.), *Insect outbreaks revisited* (pp. 91–112). Chichester: Wiley-Blackwell.

Kikuchi, Y., Hosokawa, T., & Fukatsu, T. (2007). Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. *Applied and Environmental Microbiology*, *73*(13), 4308–16.

Kirpichnikov, V. S., Illyasov, J. I., Shart, L. A., Vikhman, A. A., Ganchenko, M. V., Ostashevsky, A. L., … Tjurin, V. V. (1993). Selection of Krasnodar common carp (*Cyprinus carpio* L.) for resistance to dropsy: principal results and prospects. *Aquaculture*, *111*(1), 7–20.

Klemola, N., Klemola, T., Rantala, M. J., & Ruuhola, T. (2007). Natural host-plant quality affects immune defence of an insect herbivore. *Entomologia Experimentalis et Applicata*, *123*, 167–176.

Klemola, T., Klemola, N., Andersson, T., & Ruohomäki, K. (2007). Does immune function influence population fluctuations and level of parasitism in the cyclic geometrid moth? *Population Ecology*, *49*(2), 165–178.

Köhler, A., Pirk, C. W. W., & Nicolson, S. W. (2012). Simultaneous stressors: interactive effects of an immune challenge and dietary toxin can be detrimental to honeybees. *Journal of Insect Physiology*, *58*(7), 918–23.

Korner, P., & Schmid-Hempel, P. (2004). In vivo dynamics of an immune response in the bumble bee *Bombus terrestris*. *Journal of Invertebrate Pathology*, *87*(1), 59–66.

Korpita, T., Gómez, S., & Orians, C. M. (2014). Cues from a specialist herbivore increase tolerance to defoliation in tomato. *Functional Ecology*, *28*(2), 395–401.

Kosola, K., Dickmann, D., Paul, E., & Parry, D. (2001). Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. *Oecologia*, *129*(1), 65–74.

Krieger, R. I., Feeny, P. P., & Wilkinson, C. F. (1971). Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? *Science*, *172*(3983), 579–581.

Kukan, B. (1999). Vertical transmission of nucleopolyhedrovirus in insects. *Journal of Invertebrate Pathology*, *74*(2), 103–11.

Lambin, X., Petty, S. J., & Mackinnon, J. L. (2000). Cyclic dynamics in field vole populations and generalist predation. *Journal of Animal Ecology*, *69*, 106–118.

Langstrom, B., Tenow, O., Ericsson, A., Hellqvist, C., & Larsson, S. (1990). Effects of shoot pruning on stem growth, needle biomass, and dynamics of carbohydrates and nitrogen in Scots pine as related to season and tree age. *Canadian Journal of Forest Research*, *20*, 514–523.

Lavoie, B., & Oberhauser, K. S. (2004). Compensatory feeding in *Danaus plexippus* (Lepidoptera : Nymphalidae) in response to variation in host plant quality. *Environmental Entomology*, *33*(4), 1062–1069.

Lee, K. P., Raubenheimer, D., & Simpson, S. J. (2004). The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiological Entomology*, *29*(2), 108–117.

Lemos, F. J., & Terra, W. R. (1991). Digestion of bacteria and the role of midgut lysozyme in some insect larvae. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *100*(2), 265–268.

Linder, J. E., & Promislow, D. E. L. (2009). Cross-generational fitness effects of infection in *Drosophila melanogaster*. *Fly*, *3*(2), 143–50.

Lindroth, R. L., Hwang, S., & Osier, T. O. D. L. (1999). Phytochemical variation in quaking aspen: effects on gypsy moth susceptibility to nuclear polyhedrosis virus. *Journal of Chemical Ecology*, *25*(6), 1331–1341.

Litman, G. W., Dishaw, L. J., Cannon, J. P., Haire, R. N., & Rast, P. (2007). Alternative mechanisms of immune receptor diversity. *Current Opinion in Immunology*, *19*(5), 526–534.

Little, T. J., Connor, B. O., Colegrave, N., Watt, K., & Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology*, *13*, 489–492.

Little, T. J., Hultmark, D., & Read, A. F. (2005). Invertebrate immunity and the limits of mechanistic immunology. *Nature Immunology*, *6*(7), 651–4.

Little, T. J., & Kraaijeveld, A. R. (2004). Ecological and evolutionary implications of immunological priming in invertebrates. *Trends in Ecology & Evolution*, *19*(2), 58–60.

Lochmiller, R. L., & Deerenberg, C. (2000). Tradeoffs in evolutionary immunology: just what is the cost of immunity ? *Oikos*, *88*, 87–98.

Loker, E. S., Adema, C. M., Zhang, S.-M., & Kepler, T. B. (2004). Invertebrate immune systems--not homogeneous, not simple, not well understood. *Immunological Reviews*, *198*, 10–24.

Lorenz, L. M., & Koella, J. C. (2011). Maternal environment shapes the life history and susceptibility to malaria of *Anopheles gambiae* mosquitoes. *Malaria Journal*, *10*, 382.

Lotka, A. J. (1932). The growth of mixed populations: two species competing for a common food supply. *Journal of the Washington Academy of Sciences*, *22*, 461–469.

Lynch, M. E., Kaplan, I., Dively, G. P., & Denno, R. F. (2006). Host-plant-mediated competition via induced resistance: interactions between pest herbivores on potatoes. *Ecological Applications*, *16*(3), 855–64.

Lyytikäinen-Saarenmaa, P., & Tomppo, E. (2002). Impact of sawfly defoliation on growth of Scots pine *Pinus sylvestris* (Pinaceae) and associated economic losses. *Bulletin of Entomological Research*, *92*(2), 137–40.

Mallon, E. B., Loosli, R., & Schmid-Hempel, P. (2003). Specific versus nonspecific immune defense in the bumblebee, *Bombus terrestris* L. *Evolution*, *57*(6), 1444–1447.

Marcogliese, D. J., & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology*, *27*(3), 123–30.

Marshall, D. J. (2008). Transgenerational plasticity in the sea: context-dependent maternal effects across the life history. *Ecology*, *89*(2), 418–27.

Martemyanov, V. V, Dubovskiy, I. M., Rantala, M. J., Salminen, J. P., Belousova, I. A, Pavlushin, S. V, … Glupov, V. V. (2012). The effects of defoliation-induced delayed changes in silver birch foliar chemistry on gypsy moth fitness, immune response, and resistance to baculovirus infection. *Journal of Chemical Ecology*, *38*(3), 295–305.

Martemyanov, V. V., Bakhvalov, S. a., Dubovskiy, I. M., Glupov, V. V., Salakhutdinov, N. F., & Tolstikov, G. a. (2006). Effect of tannic acid on the development and resistance of the Gypsy moth *Lymantria dispar* L. to viral infection. *Doklady Biochemistry and Biophysics*, *409*(1), 219–222.

Martemyanov, V. V., Dubovskiy, I. M., Belousova, I. a., Pavlushin, S. V., Domrachev, D. V., Rantala, M. J., … Glupov, V. V. (2012). Rapid induced resistance of silver birch affects both innate immunity and performance of gypsy moths: the role of plant chemical defenses. *Arthropod-Plant Interactions*, (1).

Martemyanov, V. V., Dubovskiy, I. M., Belousova, I. a., Shokorova, N. S., Pavlushin, S. V., & Glupov, V. V. (2013). Sex-specific variations in gypsy moth fitness, immune function, and parasite resistance mediated by background defoliation of the host plant. *Ecological Parasitology and Immunology*, *2*, 1–7.

Mattson, M. P. (2008). Hormesis and disease resistance: activation of cellular stress response pathways. *Human & Experimental Toxicology*, *27*(2), 155–62.

Matzkin, L. M., Johnson, S., Paight, C., & Markow, T. a. (2013). Preadult parental diet affects offspring development and metabolism in *Drosophila melanogaster*. *PloS One*, *8*(3), e59530.

May, R. M. (1973). *Stability and Complexity in Model Ecosystems.* Princeton: Princeton University Press.

Mayer, A. M. (2004). Resistance to herbivores and fungal pathogens: Variations on a common theme? A review comparing the effect of secondary metabolites, induced and constitutive, on herbivores and fungal pathogens. *Israel Journal of Plant Sciences*, *52*(4), 279–292.

Mcintyre, G. S., & Gooding, R. H. (2000). Egg size, contents, and quality: maternal-age and -size effects on house fly eggs. *Canadian Journal of Zoology*, *78*, 1544–1551.

McKean, K. A, Yourth, C. P., Lazzaro, B. P., & Clark, A. G. (2008). The evolutionary costs of immunological maintenance and deployment. *BMC Evolutionary Biology*, *8*, 76.

Mckean, K. A., & Nunney, L. (2005). Bateman's principle and immunity : phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution*, *59*(7), 1510–1517.

McManus, M. L., & Csoka, G. (2007). History and impact of gypsy moth in North America and comparison to recent outbreaks in Europe. *Acta Silvatica et Lignaria Hungarica*, *3*, 47–64.

McNamara, K. B., van Lieshout, E., & Simmons, L. W. (2014). The effect of maternal and paternal immune challenge on offspring immunity and reproduction in a cricket. *Journal of Evolutionary Biology*, 1–9.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, *24*, 1161–1192.

Medzhitov, R., & Janeway, C. a. (1997). Innate immunity: the virtues of a nonclonal system of recognition. *Cell*, *91*(3), 295–8.

Messina, F. J., & Slade, A. F. (1999). Expression of a life-history trade-off in a seed beetle depends on environmental context. *Physiological Entomology*, *24*(4), 358–363.

Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, *16*(5), 254–260.

Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. a. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, *20*(12), 685–92.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings. Biological Sciences / The Royal Society*, *272*(1581), 2601–7.

Moreau, J., Martinaud, G., Troussard, J.-P., Zanchi, C., & Moret, Y. (2012). Trans-generational immune priming is constrained by the maternal immune response in an insect. *Oikos*, *121*(11), 1828–1832.

Moret, Y. (2006). “Trans-generational immune priming”: specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proceedings. Biological Sciences / The Royal Society*, *273*(1592), 1399–405.

Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science*, *290*(5494), 1166–1168.

Moret, Y., & Siva-Jothy, M. T. (2003). Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proceedings. Biological Sciences / The Royal Society*, *270*(1532), 2475–80.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, *13*(10), 403–407.

Mowlds, P., Barron, A., & Kavanagh, K. (2008). Physical stress primes the immune response of *Galleria mellonella* larvae to infection by *Candida albicans*. *Microbes and Infection / Institut Pasteur*, *10*(6), 628–34.

Müller, T., Müller, M., Behrendt, U., & Stadler, B. (2003). Diversity of culturable phyllosphere bacteria on beech and oak: the effects of lepidopterous larvae. *Microbiological Research*, *158*(4), 291–7.

Murray, K. D., & Elkinton, J. S. (1989). Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). *Journal of Invertebrate Pathology*, *53*(3), 324–334.

Myers, J. H. (1988). Can a general hypothesis explain population cycles of forest lepidoptera? *Advances in Ecological Research*, *18*, 179–232.

Myers, J. H. (2000). Population fluctuations of the western tent caterpillar in southwestern British Columbia. Population Ecology, 42(3), 231-241.

Myers, J. H., & Cory, J. S. (2013). Population cycles in forest lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 131025103132004.

Myers, J. H., Cory, J. S., Ericsson, J. D., & Tseng, M. L. (2011). The effect of food limitation on immunity factors and disease resistance in the western tent caterpillar. *Oecologia*, *167*(3), 647–55.

Myers, J. H., & Kuken, B. (1995). Changes in the fecundity of tent caterpillars : a correlated character of disease resistance or sublethal effect of disease ? *Oecologia*, *103*, 475–480.

Myers, J. H., & Williams, K. S. (1984). Does tent caterpillar attack reduce the food quality of red alder foliage ? *Oecologia*, *62*(1), 74–79.

Myers, J. H., & Williams, K. S. (1987). Lack of short or long term inducible defenses in the red alder - western tent caterpillar system. *Oikos*, *48*(1), 73–78.

Nätt, D., Rubin, C.-J., Wright, D., Johnsson, M., Beltéky, J., Andersson, L., & Jensen, P. (2012). Heritable genome-wide variation of gene expression and promoter methylation between wild and domesticated chickens. *BMC Genomics*, *13*(1), 59.

Nishida, R. (2002). Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology*, *47*, 57–92.

Nykänen, H., & Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance : a meta-analysis. *Oikos*, *104*, 247–268.

O'Brien, D. M., Fogel, M. L., & Boggs, C. L. (2002). Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(7), 4413–8.

Osborne, Jason (2002). Notes on the use of data transformations. *Practical Assessment, Research & Evaluation, 8*(6), 1-8..

Osier, T. L., Hwang, S., & Lindroth, R. L. (2000). Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. *Ecological Entomology*, *25*, 197–207.

Ossipov, V., Haukioja, E., Ossipova, S., Hanhimäki, S., & Pihlaja, K. (2001). Phenolic and phenolic-related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. *Biochemical Systematics and Ecology*, *29*(3), 223–240.

Ostfeld, R. S., Keesing, F., & Eviner, V. T. (Eds.). (2010). *Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems.* Princeton University Press.

Parry, D., Herms, D. A., & Mattson, W. J. (2003). Responses of an insect folivore and its parasitoids to multiyear experimental defoliation of aspen. *Ecology*, *84*(7), 1768–1783.

Piene, H., Ostaff, D. P., & Eveleigh, E. S. (2001). Growth loss and recovery following defoliation by the balsam fir sawfly in young, spaced balsam fir stands. *The Canadian Entomologist*, *133*(5), 675–686.

Pieterse, C. M. J., & Dicke, M. (2007). Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends in Plant Science*, *12*(12), 564–9.

Plaistow, S. J., Lapsley, C. T., & Benton, T. G. (2006). Context-dependent intergenerational effects : the interaction between past and present environments and Its effect on population dynamics. *The American Naturalist*, *167*(2), 206–215.

Plowright, R. K., Sokolow, S. H., Gorman, M. E., Daszak, P., & Foley, J. E. (2008). Causal inference in disease ecology: investigating ecological drivers of disease emergence. *Frontiers in Ecology and the Environment*, *6*(8), 420–429.

Poulin, R., & Thomas, F. (2008). Epigenetic effects of infection on the phenotype of host offspring: parasites reaching across host generations. *Oikos*, *117*(3), 331–335.

Povey, S., Cotter, S. C., Simpson, S. J., Lee, K. P., & Wilson, K. (2009). Can the protein costs of bacterial resistance be offset by altered feeding behaviour? *The Journal of Animal Ecology*, *78*(2), 437–46.

Pöykkö, H., & Mänttäri, S. (2012). Egg size and composition in an ageing capital breeder - consequences for offspring performance. *Ecological Entomology*, *37*(5), 330–341.

Raffel, T. R., Martin, L. B., & Rohr, J. R. (2008). Parasites as predators: unifying natural enemy ecology. *Trends in Ecology & Evolution*, *23*(11), 610–8.

Rantala, M. J., & Roff, D. a. (2005). An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Functional Ecology*, *19*(2), 323–330.

Räsänen, K., & Kruuk, L. E. B. (2007). Maternal effects and evolution at ecological time-scales. *Functional Ecology*, *21*(3), 408–421.

Raubenheimer, D., & Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Animal Behavior*, *45*, 953–964.

Raymond, B., Sayyed, A. H., & Wright, D. J. (2006). The compatibility of a nucleopolyhedrosis virus control with resistance management for *Bacillus thuringiensis*: co-infection and cross-resistance studies with the diamondback moth, *Plutella xylostella*. *Journal of Invertebrate Pathology*, *93*(2), 114–20.

Reynolds, J. J. H., White, A., Sherratt, J. a, & Boots, M. (2011). The population dynamical consequences of density-dependent prophylaxis. *Journal of Theoretical Biology*, *288*, 1–8.

Rohr, J. R., Elskus, A. A., Shepherd, B. S., Crowley, P. H., McCarthy, T. M., Niedzwiecki, J. H., … Palmer, B. D. (2004). Multiple stressors and salamanders: effects of an herbicide, food limitation, and hydroperiod. *Ecological Applications*, *14*(4), 1028–1040.

Rooke, T., & Bergström, R. (2006). Growth, chemical responses and herbivory after simulated leaf browsing in *Combretum apiculatum*. *Plant Ecology*, *189*(2), 201–212.

Rossiter, M. C. (1991a). Environmentally-based maternal effects: a hidden force in insect population dynamics? *Oecologia*, *87*(2), 288–294.

Rossiter, M. C. (1991b). Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. *Functional Ecology*, *5*, 386–393.

Rossiter, M. C. (1994). Maternal effects hypothesis of herbivore outbreak: a framework for the inclusion of population-quality variables as central features of herbivore population-dynamics models. *Bioscience*, *44*(11), 752–763.

Rossiter, M. C., Schultz, J. C., & Baldwin, I. T. (1988). Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology*, *69*(1), 267–277.

Rossiter, M., Cox-foster, D., Abou-zaid, M. M., & Bergeron, D. (1996). Egg protein insolubility in *Lymantria dispar* versus other forest Lepidoptera. *Chemoecology*, *84*, 74–84.

Rotem, K., Agrawal, A. A., & Kott, L. (2003). Parental effects in *Pieris rapae* in response to variation in food quality : adaptive plasticity across generations ? *Ecological Entomology*, *28*, 211–218.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. *The Journal of Animal Ecology*, *79*(2), 403–13.

Roth, O., Sadd, B. M., Schmid-Hempel, P., & Kurtz, J. (2009). Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. *Proceedings. Biological Sciences / The Royal Society*, *276*(1654), 145–51.

Rothman, L. D. (1997). Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. *Ecology*, *78*(5), 1481–1493.

Rothman, L. D., & Myers, J. H. (1994). Nuclear polyhedrosis virus treatment effect on reproductive potential of western tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, *23*(4), 864–869.

Rothman, L., & Myers, J. H. (1996). Debilitating effects of viral diseases on host lepidoptera. *Journal of Invertebrate Pathology*, *67*(1), 1–10.

Rothman, L., & Myers, J. H. (1996). Is fecundity correlated with resistance to viral disease in the western tent caterpillar? *Ecological Entomology*, *21*, 396–398.

Rowley, A. F., & Powell, A. (2007). Invertebrate immune systems specific, quasi-specific, or nonspecific? *Journal of Immunology (Baltimore, Md. : 1950)*, *179*(11), 7209–14.

Roy, H. E., Steinkraus, D. C., Eilenberg, J., Hajek, a E., & Pell, J. K. (2006). Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology*, *51*, 331–57.

Ryu, J.-H., Kim, S.-H., Lee, H.-Y., Bai, J. Y., Nam, Y.-D., Bae, J.-W., … Lee, W.-J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science (New York, N.Y.)*, *319*(5864), 777–82.

Saastamoinen, M., Hirai, N., & van Nouhuys, S. (2013). Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. *Oecologia*, *171*(1), 93–104.

Sadd, B. M., Kleinlogel, Y., Schmid-Hempel, R., & Schmid-Hempel, P. (2005). Trans-generational immune priming in a social insect. *Biology Letters*, *1*(4), 386–8.

Sadd, B. M., & Schmid-Hempel, P. (2006). Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current Biology : CB*, *16*(12), 1206–10.

Sadd, B. M., & Schmid-Hempel, P. (2007). Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Current Biology : CB*, *17*(24), R1046–7.

Sadd, B. M., & Schmid-Hempel, P. (2009). A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumble-bees. *Biology Letters*, *5*(6), 798–801.

Saejeng, a, Tidbury, H., Siva-Jothy, M. T., & Boots, M. (2010). Examining the relationship between hemolymph phenoloxidase and resistance to a DNA virus, *Plodia interpunctella* granulosis virus (PiGV). *Journal of Insect Physiology*, *56*(9), 1232–6.

Sarfraz, R. M., Cory, J. S., & Myers, J. H. (2013). Life-history consequences and disease resistance of western tent caterpillars in response to localised, herbivore-induced changes in alder leaf quality. *Ecological Entomology*, *38*(1), 61–67.

Schaller, A. (Ed.). (2008). *Induced Plant Resistance to Herbivory*. Springer.

Schlotz, N., Ebert, D., & Martin-Creuzburg, D. (2013). Dietary supply with polyunsaturated fatty acids and resulting maternal effects influence host--parasite interactions. *BMC Ecology*, *13*(1), 41.

Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proceedings. Biological Sciences / The Royal Society*, *270*(1513), 357–66.

Schmid-Hempel, P. (2005). Natural insect host-parasite systems show immune priming and specificity: puzzles to be solved. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *27*(10), 1026–34.

Selye, H. (1950). Stress and the general adaptation syndrome*. *British Medical Journal*, *1*(4667), 1383–1392.

Shelby, K. S., & Popham, H. J. R. (2006). Plasma phenoloxidase of the larval tobacco budworm, *Heliothis virescens*, is virucidal. *Journal of Insect Science (Online)*, *6*(13), 1–12.

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.

Shikano, I., Ericsson, J. D., Cory, J. S., & Myers, J. H. (2010). Indirect plant-mediated effects on insect immunity and disease resistance in a tritrophic system. *Basic and Applied Ecology*, *11*(1), 15–22.

Shikano, I., Halpert-Scanderbeg, O., Oak, M., & Cory, J. S. (2014). Trade-offs between trans-generational transfer of nutritional stress tolerance and immune priming. Manuscript submitted for publication.

Shoemaker, K. L., & Adamo, S. a. (2007). Adult female crickets, *Gryllus texensis*, maintain reproductive output after repeated immune challenges. *Physiological Entomology*, *32*(2), 113–120.

Sih, A., Englund, G., & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution*, *13*(9), 350–355.

Simpson, S. J., & Simpson, C. L. (1990). The mechanisms of nutritional compensation by phytophagous insects. *Insect-Plant Interactions*, *2*, 111–160.

Singer, M. S., Mace, K. C., & Bernays, E. a. (2009). Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. *PloS One*, *4*(3), e4796.

Siva-Jothy, M. T., & Thompson, J. J. W. (2002). Short-term nutrient deprivation affects immune function. *Physiological Entomology*, *27*, 206–212.

Smith, E. M., Hoi, J. T., Eissenberg, J. C., Shoemaker, J. D., Neckameyer, W. S., Ilvarsonn, A. M., … Zempleni, J. (2007). Feeding *Drosophila* a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns. *The Journal of Nutrition*, *137*(9), 2006–12.

Sparks, W. O., Bartholomay, L., & Bonning, B. C. (2008). Insect immunity to viruses. In *Insect Immunology* (pp. 209–242).

Stadler, B., & Müller, T. (2000). Effects of aphids and moth caterpillars on epiphytic microorganisms in canopies of forest trees. *Canadian Journal for Resources*, *30*, 631–638.

Stehr, F. W., & Cook, E. F. (1968). A revision of the genus Malacosoma Hubner in North America (Lepidoptera: Lasiocampidae): systematics, biology, immatures, and parasites. *US National Museum,* (Bulletin 276).

Stout, M. J., Thaler, J. S., & Thomma, B. P. H. J. (2006). Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annual Review of Entomology*, *51*, 663–89.

Swaby, A. N. (2013). *Parental provisioning in the face of pathogenic pressures : contributions to offspring in a social insect , the dampwood termite (Zootermopsis angusticollis). (Master's thesis)*. Retreived from http://iris.lib.neu.edu/biology_theses/23/

Tammaru, T., & Haukioja, E. (1996). Capital breeders and income breeders among Lepidoptera - consequences to population dynamics. *Oikos*, *77*(3), 561–564.

Tammaru, T., Kaitaniemi, P., & Ruohomäki, K. (1996). Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): relation to body size and consequences to population dynamics. *Oikos*, *77*(3), 407–416.

Terenius, O. (2008). Hemolin-A lepidopteran anti-viral defense factor? *Developmental and Comparative Immunology*, *32*(4), 311–6.

Terenius, O., Bettencourt, R., Lee, S. Y., Li, W., Söderhäll, K., & Faye, I. (2007). RNA interference of Hemolin causes depletion of phenoloxidase activity in *Hyalophora cecropia*. *Developmental and Comparative Immunology*, *31*(6), 2007.

Tidbury, H. J., Pedersen, A. B., & Boots, M. (2011). Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings. Biological Sciences / The Royal Society*, *278*(1707), 871–6.

Torres-vila, L. M., & Rodriguez-Molina, M. C. (2002). Egg size variation and its relationship with larval performance in the Lepidoptera : the case of the European grapevine moth *Lobesia botrana*. *Oikos*, *99*, 272–283.

Trauer, U., & Hilker, M. (2013). Parental legacy in insects: variation of transgenerational immune priming during offspring development. *PloS One*, *8*(5), e63392.

Triggs, A., & Knell, R. J. (2012a). Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. *The Journal of Animal Ecology*, *81*(2), 386–94.

Triggs, A. M., & Knell, R. J. (2012b). Parental diet has strong transgenerational effects on offspring immunity. *Functional Ecology*, *26*(6), 1409–1417.

Trudeau, D., Washburn, J. O., & Volkman, L. E. (2001). Central Role of Hemocytes in *Autographa californica* M Nucleopolyhedrovirus Pathogenesis in *Heliothis virescen*s and Helicoverpa zea *Journal of Virology*, *75*(2), 996–1003.

Trumble, J. T., Kolodny-Hirsch, D. M., & Ting, I. P. (1993). Plant Compensation for Arthropod Herbivory. *Annual Review of Entomology*, *38*, 93–119.

Tuomi, J., Niemela, P., Haukioja, E., Siren, S., & Neuvonen, S. (1984). Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia*, *61*, 208–210.

Tyler, E. R., Adams, S., & Mallon, E. B. (2006). An immune response in the bumblebee, *Bombus terrestris* leads to increased food consumption. *BMC Physiology*, *6*, 6.

Underwood, N. (1999). The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist*, *153*(3), 282–294.

Underwood, N. (2010). Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. *Oikos*, *119*(12), 1993–1999.

Valtonen, T. M., Kangassalo, K., Pölkki, M., & Rantala, M. J. (2012). Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. *PloS One*, *7*(2), e31611.

Valtonen, T. M., Kleino, A., Rämet, M., & Rantala, M. J. (2010). Starvation reveals maintenance cost of humoral immunity. *Evolutionary Biology*, *37*(1), 49–57.

Van Frankenhuyzen, K., Ebling, P., Thurston, G., Lucarotti, C., Royama, T., Guscott, R., … Silver, J. (2002). Incidence and impact of *Entomophaga aulicae* (Zygomycetes: Entomophthorales) and a nucleopolyhedrovirus in an outbreak of the whitemarked tussock moth (Lepidoptera:Lymantriidae). *The Canadian Entomologist*, *134*, 825–845.

Vandegehuchte, M. B., Lemière, F., Vanhaecke, L., Vanden Berghe, W., & Janssen, C. R. (2010). Direct and transgenerational impact on *Daphnia magna* of chemicals with a known effect on DNA methylation. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology : CBP*, *151*(3), 278–85.

Vilmos, P., & Kurucz, E. (1998). Insect immunity: evolutionary roots of the mammalian innate immune system. *Immunology Letters*, *62*(2), 59–66.

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., … Lemaitre, B. (2005). *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(32), 11414–9.

Volterra, V. (1926). Variations and fluctuations of the numbers of individuals in animal species living together. *Journal Du Conseil Permanent International Pour l'Exploration de La Mer*, *3*, 3–51.

Vorburger, C., Gegenschatz, S. E., Ranieri, G., & Rodriguez, P. (2008). Limited scope for maternal effects in aphid defence against parasitoids. *Ecological Entomology*, *33*(2), 189–196.

Washburn, J. O., Haas-Stapleton, E. J., Tan, F. F., Beckage, N. E., & Volkman, L. E. (2000). Co-infection of *Manduca sexta* larvae with polydnavirus from *Cotesia congregata* increases susceptibility to fatal infection by *Autographa californica* M Nucleopolyhedrovirus. *Journal of Insect Physiology*, *46*(2), 179–190.

Washburn, J. O., Kirkpatrick, B. A., Haas-stapleton, E., & Volkman, L. E. (1998). Evidence that the stilbene-derived optical brightener M2R enhances *Autographa californica* M Nucleopolyhedrovirus infection of *Trichoplusia ni* and *Heliothis virescens* by preventing sloughing of infected midgut epithelial cells. *Biological Control*, *69*(11), 58–69.

Washburn, J. O., Kirkpatrick, B. A., & Volkman, L. E. (1995). Comparative pathogenesis of *Autographa californica* M nuclear polyhedrosis virus in larvae of *Trichoplusia ni* and *Heliothis virescens*. Virology, 209, 561–568

Washburn, J. O., Kirkpatrick, B. A., & Volkman, L. E. (1996). Insect protection against viruses. *Nature*, *383*, 767.

Weiss, B., & Aksoy, S. (2011). Microbiome influences on insect host vector competence. *Trends in Parasitology*, *27*(11), 514–22.

Wellington, W. G. (1957). Individual differences as a factor in population dynamics: the development of a problem. *Canadian Journal of Zoology*, *35*, 293–323.

Wellington, W. G. (1960). Qualitative changes in natural populations during changes in abundance. *Canadian Journal of Zoology*, *38*, 289–314.

Wellington, W. G. (1964). Qualitative changes in populations in unstable environments. *The Canadian Entomologist*, *96*(1-2), 436–451.

Wellington, W. G. (1965). Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). *The Canadian Entomologist*, *97*(1), 1–14.

Wheeler, G. S., Slansky, F., & Yu, S. J. (2001). Food consumption, utilization and detoxification enzyme activity of larvae of three polyphagous noctuid moth species when fed the botanical insecticide rotenone. *Entomologia Experimentalis et Applicata*, *98*(2), 225–239.

Williams, H. L., Monge-Monge, K. S., Otvos, I. S., Reardon, R., & Ragenovich, I. (2011). Genotypic variation among Douglas-fir tussock moth nucleopolyhedrovirus (OpNPV) isolates in the western United States. *Journal of Invertebrate Pathology*, *108*(1), 13–21.

Wilson, K., Cotter, S. C., Reeson, A. F., & Pell, J. K. (2001). Melanism and disease resistance in insects. *Ecology Letters*, *4*, 637–649.

Wilson, K., & Reeson, A. F. (1998). Density-dependent prophylaxis: evidence from Lepidoptera–baculovirus interactions? *Ecological Entomology*, *23*, 100–101.

Wilson, K., Thomas, M. B., Blanford, S., Doggett, M., Simpson, S. J., & Moore, S. L. (2002). Coping with crowds: density-dependent disease resistance in desert locusts. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(8), 5471–5.

Wittstock, U., & Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology, 5(4), 300–7.

Woods, S. A., & Elkinton, J. S. (1987). Biomodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. *Journal of Invertebrate Pathology*, *50*(2), 151–157.

Xiang, H., Zhu, J., Chen, Q., Dai, F., Li, X., Li, M., … Wang, J. (2010). Single base-resolution methylome of the silkworm reveals a sparse epigenomic map. *Nature Biotechnology*, *28*(5), 516–20.

Yadav, R. K. P., Papatheodorou, E. M., Karamanoli, K., Constantinidou, H.-I. a., & Vokou, D. (2008). Abundance and diversity of the phyllosphere bacterial communities of Mediterranean perennial plants that differ in leaf chemistry. *Chemoecology*, *18*(4), 217–226.

Yang, S., Ruuhola, T., & Rantala, M. J. (2007). Impact of starvation on immune defense and other life- history traits of an outbreaking geometrid , *Epirrita autumnata* : a possible causal trigger for the crash phase of population cycle. *Annual Zoology Fennici*, *44*, 89–96.

Young, S. Y., Yang, J. G., & Felton, G. W. (1995). Inhibitory effects of dietary tannins on the infectivity of a nuclear polyhedrosis virus to *Helicoverpa zea* (Noctuidae: Lepidoptera). *Biological Control*, *5*, 145–150.

Zanchi, C., Troussard, J.-P., Moreau, J., & Moret, Y. (2012). Relationship between maternal transfer of immunity and mother fecundity in an insect. *Proceedings. Biological Sciences / The Royal Society*, *279*(1741), 3223–30.

Chapter 3. Conclusion

In this thesis, I set out to test the effects of single and multiple dietary stressors on transgenerational disease resistance, immunity, and life history traits in the WTC. The results from this study provide the first example of transgenerational effects on pathogen resistance and immunity from a parental diet of induced foliage. Although more subtle than the effects of plant induction, the consumption of non-pathogenic foliar bacteria by parents also affected offspring virus resistance and fitness in novel and unexpected ways. It was originally hypothesized that combinations of stressors would negatively affect offspring immunocompetence and overall condition in a synergistic manner, resulting in enhanced virus susceptibility and providing a possible trigger for the onset of viral epizootics in this species. Contrary to predictions, combinations of stressors (induced foliage and leaf bacteria) had an additive, protective effect on offspring exposed to virus.

The results of this study underline the importance of dietary transgenerational effects in modulating the host-pathogen relationship and emphasize the need for further investigation into several areas where our understanding is limited. Firstly, we need to assess the extent to which these results represent generalities through determining the mechanisms behind the enhanced virus resistance and immunity in the offspring of induced-foliage-fed insects. More information is also needed on the nature of the relationship between foliar bacteria and insect herbivores, and how or whether these might interact with other bacteria, for example, within the insect gut. Food limitation is clearly an important stressor in many herbivores and realistic experiments need to be carried out to evaluate the potential for transgenerational effects arising from the interaction between food limitation and other stressors. More broadly, insects can be infected by many pathogens, most of which have received little attention, and the diversity and influence of this entomopathogen community on individuals and populations could be a rich area for research. Finally, we need to evaluate how stressor-

induced trade-offs between parental fitness traits relate to offspring quality, the potential for life-stage-dependent transgenerational effects, and how the results of this study more broadly relate to population cycles in forest Lepidoptera and other taxa. Below, I briefly consider these aspects and how we might go about answering the questions posed by this study.

As discussed earlier in Chapter 2.B, the mechanisms behind transgenerational effects on virus resistance and immunocompetence from a parental diet of induced foliage are unknown. Were the transgenerational effects from induced foliage the result of specific changes in leaf secondary chemicals or nutritional quality, or did parents "perceive" foliage from attacked branches as more general cues for increased disease risk, provisioning offspring in an adaptive, plastic manner (Mousseau & Fox, 1998; Hunter, 2002; Räsänen & Kruuk, 2007)? In order to get at the mechanisms and adaptive potential for enhanced offspring resistance and fitness trade-offs from induced foliage, further experiments should delve into the importance of specific plant chemicals at varying concentrations and examine the effect of host plant identity in a transgenerational context. Were the cross-generational effects from induced foliage the result of changes to provisioning of yet unknown egg components or were they due to epigenetic changes? One way to investigate this would be to look for gender-specific transgenerational effects by separating the effects of female and male contributions to offspring, as has been done in previous studies (see Roth et al., 2010; Triggs & Knell, 2012). At the molecular level, detection of specific DNA methylations and differential gene expression can begin to reveal the potential role of epigenetics in systems like the WTC (see Elango et al., 2009; Xiang et al., 2010).

The results from this study suggest that realistic levels of non-pathogenic leaf-associated bacteria, taken in through natural routes, can have significant transgenerational effects on fitness in the WTC. Future ecological studies should attempt to use realistic methods for introducing bacteria (as opposed to injections and/or extremely high bacterial concentrations). The effects of non-pathogenic bacteria should be evaluated across a range of concentrations, allowing for the assessment of threshold and dose-dependent effects. It would also be interesting to examine whether coating a leaf surface in varying concentrations of bacteria provides any additional or otherwise

modified nutritive value to larvae, and whether any such nutrition boost could affect life history and/or immune function. This could be explored through experiments involving feeding insects live bacteria, heat-killed bacteria, or an artificial formulation based on a nutritional analysis of bacteria. Moreover, further research could assess the potential for foliar bacteria interacting with or functioning as gut microbes (Dillon & Dillon, 2004; Broderick et al., 2004; Indiragandhi et al., 2007; Broderick et al., 2009; Indiragandhi et al., 2011). One way to establish the potential relationship between foliar bacteria and gut microbiota would be to test the fitness consequences of adding antibiotics to diets with or without foliar bacteria (similar to the methodology used by Broderick et al., 2009).

It is also important for future research to delve into the real-world presence and effects of leaf microbiota. The bacterial species used in this experiment were chosen on the basis that they were commonly found on deciduous leaf surfaces in North America (Hirano & Upper, 2000; Müller et al., 2003; Redford & Fierer, 2009). It would be interesting to investigate whether the transgenerational effects observed in this study are dependent on the identity of bacterial species, as well as their relative diversity. One way in which this could be explored in relation to population density would be to collect foliage samples from sites varying in larval density and quantifying species and relative abundances of foliar bacteria. Such field-based data could be accompanied by controlled lab studies testing the effect of bacterial diversity/abundance on fitness traits and disease susceptibility within and across generations.

Further work is needed to evaluate the potential for synergistic transgenerational effects arising from the interaction between food limitation and other stressors. The high mortality and potential selection in starved parents was in the same direction as the effects from other stressors and agrees with previous studies that suggest a transgenerational increase in disease resistance (Myers et al., 2011; Boots & Roberts, 2012; Shikano et al., in press). Regardless, we require further information on the subtle effects of partial starvation in the absence of high mortality and selective pressures (Saastamoinen et al., 2013; Shikano et al., in press). However, it remains that food limitation can be severe in natural situations – perhaps the high parental mortality and possible selection I observed from starvation of parents is an integral part of the scenario seen in peak, high-density populations of the WTC, as well as in other systems.

Little is known about pathogen communities in insects. Pathogens can often be carried sublethally and can affect hosts in subtle and chronic ways, making a comprehensive understanding of these relationships difficult and invariably complex. The WTC is infected by a range of entomopathogens, although McplNPV is the primary pathogen associated with population declines (Fitzgerald, 1995; Myers, 2000; Cory & Myers, 2009; Myers & Cory, 2013). While baculoviruses have been the main pathogen group of interest in several studies involving forest Lepidopterans, it is important to examine the influence of the broader pathogen community on species such as the WTC. Unidentified pathogens, including species of bacteria and fungi, have been noted as significant mortality agents in previous WTC bioassays (Cory & Myers, 2009; Sarfraz et al, 2013) and may be important pathogens of this species, especially in stressful environments. Interactions between pathogens could be important, and their expression could be dependent on insect condition. Pathogens and other microorganisms need to be identified by techniques such as PCR/RT-PCR, and through genomic techniques (Kukan & Myers, 1995; Crampton et al., 1997; Call et al., 2003; Entz et al., 2005; Behura, 2006; Thorne et al., 2007). Once pathogens are identified and patterns of co-occurrence are studied, attempts to isolate specific microbes could be made, followed by bioassays testing intra- and transgenerational effects of diet on disease resistance. Additionally, investigation into the potential for sublethal or vertically transmitted infections could provide insight into the pervasiveness of specific entomopathogens (Goulson & Cory, 1995; Mangin et al., 1995; Burden et al., 2002; Quesada-Moraga et al., 2004; Vizoso & Ebert, 2004).

The lab-based simulation of multiple stressors (i.e. diet treatments) experienced in high-density WTC populations was evaluated against changes in life history traits in the parental generation, although it was not logistically feasible to measure the impact of the treatments on parental disease resistance or immunological parameters. The study of these aspects would provide a baseline for within generational effects of dietary factors, and allow for a better understanding of how dietary factors may trigger changes in resource allocation, leading to potential trade-offs. How trade-offs and immune responses in invertebrates compare between parents and offspring is largely unknown at this point; evidence points towards both similar (Triggs & Knell, 2012) and distinct responses (Freitak et al., 2007, 2009a). Through testing both generations, the strength,

direction (positive or negative) and adaptive value of transgenerational effects may be more accurately assessed.

The existence and adaptive value of dietary transgenerational effects might relate to life history, in particular generation time; in univoltine species, such as the WTC, conditions for subsequent generations may be less predictable versus multivoltine species, which experience more frequent generations and a greater chance for "matching" environmental conditions between parent and offspring (Mitchell & Read, 2005; Plaistow & Benton, 2009; Boots & Roberts, 2012; Hoyle & Ezard, 2012). Another area worth considering is how fitness trade-offs and resource provisioning to offspring differ between income breeders (i.e. adults feed and are able to supplement energy/nutrient reserves accumulated in the larval stage; e.g. the monarch butterfly, *D. plexippus*) and capital breeders (i.e. species for which the larval stage completely determines the resource pool available for adult functions and reproduction; e.g. the WTC). Capital breeding is a strategy shared among many outbreaking Lepidopteran species (Tammaru & Haukioja, 1996); density-related dietary effects may be especially relevant for population dynamics in these species as opposed to income breeders, where adult feeding may serve to offset stressors experienced as larvae (Tammaru et al., 2004; Boggs & Freeman, 2005).

As considered in the discussion of Chapter 2.B, I suspected that trade-offs in offspring growth and immune function might have occurred at different developmental stages, depending on parental dietary factors. Pathogen assays and immune measures of offspring during different life stages may allow for the assessment of the compensatory growth hypothesis outlined in this study (similar to the experimental design of Trauer & Hilker, 2013). Such an exploration would also shed light onto the ecological and evolutionary significance of transgenerational effects in the WTC. While transgenerational effects are usually considered as bifurcated responses (i.e. positive or negative in direction) (Falconer, 1965; Janssen et al., 1988; Lande & Price, 1989; Hoyle & Ezard, 2012), evidence for reversals on life history traits over the juvenile development period in WTC offspring hints that the direction of transgenerational effects is both context *and* stage dependent.

Considerable gaps exist in our understanding of population cycles and formulating general hypotheses that can explain outbreaks in forest Lepidopterans remains a challenge. Although individual quality and susceptibility to disease are likely important drivers of cyclic patterns, there is still little known about how environmental factors influence fitness and immunocompetence, especially across generations and at the population scale. Further integration of lab-based studies investigating the role of diet on immune function and field-based studies tracking density-associated environmental conditions in relation to host susceptibility and disease prevalence are necessary to progress in this direction. Additionally, studies incorporating multiple top-down and bottom-up influences will be key for a greater understanding of cyclic population dynamics (Myers & Cory, 2013).

In this thesis, I demonstrated the existence of dietary transgenerational effects on disease resistance, immunity, and life history traits of the WTC. The results from this study pose new questions regarding the role of environmental heterogeneity on population cycles of forest Lepidoptera and the complex, context dependent nature of the host/pathogen relationship.

3.A. References

Behura, S. K. (2006). Molecular marker systems in insects: current trends and future avenues. *Molecular Ecology*, *15*(11), 3087–113.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, *144*(3), 353–61.

Boots, M., & Roberts, K. E. (2012). Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proceedings. Biological Sciences / The Royal Society*, *279*(1744), 4009–14.

Broderick, N. A, Robinson, C. J., McMahon, M. D., Holt, J., Handelsman, J., & Raffa, K. F. (2009). Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biology*, *7*, 11.

Broderick, N. A., Raffa, K. F., Goodman, R. M., & Handelsman, J. (2004). Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology*, *70*(1), 293–300.

Burden, J. P., Griffiths, C. M., Cory, J. S., Smith, P., & Sait, S. M. (2002). Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Molecular Ecology*, *11*(3), 547–55.

Call, D. R., Borucki, M. K., & Loge, F. J. (2003). Detection of bacterial pathogens in environmental samples using DNA microarrays. *Journal of Microbiological Methods*, *53*(2), 235–243.

Cory, J. S., & Myers, J. H. (2009). Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis. *The Journal of Animal Ecology*, *78*(3), 646–55.

Crampton, J. M., Beard, C. B., & Louis, C. (1997). *The molecular biology of insect disease vectors: a methods manual*. Chapman & Hall.

Dillon, R. J., & Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology*, *49*(98), 71–92.

Elango, N., Hunt, B. G., Goodisman, M. a D., & Yi, S. V. (2009). DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. Proceedings of the National Academy of Sciences of the United States of America, 106(27), 11206–11.

Entz, S. C., Johnson, D. L., & Kawchuk, L. M. (2005). Development of a PCR-based diagnostic assay for the specific detection of the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum*. *Mycological Research*, *109*(11), 1302–1312.

Falconer, D. S. (1965). Maternal effects and selection response. *Genetics Today*, *3*, 763–774.

Fitzgerald, T. D. (1995). *The Tent Caterpillars*. New York: Cornell University Press.

Freitak, D., Heckel, D. G., & Vogel, H. (2009a). Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (*Trichoplusia ni*). *Frontiers in Zoology*, *6*, 7.

Freitak, D., Heckel, D. G., & Vogel, H. (2009b). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings. Biological Sciences / The Royal Society*, *276*(1667), 2617–24.

Freitak, D., Wheat, C. W., Heckel, D. G., & Vogel, H. (2007). Immune system responses and fitness costs associated with consumption of bacteria in larvae of Trichoplusia ni. *BMC Biology*, *5*, 56.

Goulson, D., & Cory, J. S. (1995). Sublethal effects of baculovirus in the cabbage moth, *Mamestra brassicae*. *Biological Control*, *5*, 361–367.

Hirano, S. S., & Upper, C. D. (2000). Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* — a pathogen, ice nucleus, and epiphyte. *Microbiology and Molecular Biology Reviews*, *64*(3), 624–653.

Hoyle, R. B., & Ezard, T. H. G. (2012). The benefits of maternal effects in novel and in stable environments. *Journal of the Royal Society, Interface / the Royal Society*, *9*(75), 2403–13.

Hunter, M. D. (2002). Maternal effects and the population dynamics of insects on plants. *Agricultural and Forest Entomology*, *4*(1), 1–9.

Iasur-Kruh, L., Weintraub, P. G., Mozes-Daube, N., Robinson, W. E., Perlman, S. J., & Zchori-Fein, E. (2013). Novel Rickettsiella bacterium in the leafhopper *Orosius albicinctus* (Hemiptera: Cicadellidae). *Applied and Environmental Microbiology*, *79*(14), 4246–52.

Indiragandhi, P., Anandham, R., Madhaiyan, M., Poonguzhali, S., Kim, G. H., Saravanan, V. S., & Sa, T. (2007). Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, *Plutella xylostella* and their potential for, antagonism towards entomopathogenic fungi and host insect nutrition. *Journal of Applied Microbiology*, *103*(6), 2664–75.

Indiragandhi, P., Anandham, R., & Sa, T. M. (2011). Functional Significance of Insect Gut Bacteria and Their Role in Host Insect Processes, Development, and Crop Production. In D. K. Maheshwari (Ed.), *Bacteria in Agrobiology: Plant Growth Responses*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Janssen, G. M., de Jong, G., Joosse, E. N. G., & Scharloo, W. (1988). A negative maternal effect in springtails. *Evolution*, *42*(4), 828–834.

Kikuchi, Y., Hosokawa, T., Nikoh, N., Meng, X.-Y., Kamagata, Y., & Fukatsu, T. (2009). Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *BMC Biology*, *7*, 2.

Klemola, T., Andersson, T., & Ruohomäki, K. (2014). Delayed density-dependent parasitism of eggs and pupae as a contributor to the cyclic population dynamics of the autumnal moth. *Oecologia*.

Kukan, B., & Myers, J. H. (1995). DNA hybridization assay for detection of nuclear polyhedrosis virus in tent caterpillars. *Journal of Invertebrate Pathology*, *66*, 231–236.

Mangin, K. L., Lipsitch, M., & Ebert, D. (1995). Virulence and transmission modes of two microsporidia in *Daphnia magna*. *Parasitology*, *111*, 133–142.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings. Biological Sciences / The Royal Society*, *272*(1581), 2601–7.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, *13*(10), 403–407.

Müller, T., Müller, M., Behrendt, U., & Stadler, B. (2003). Diversity of culturable phyllosphere bacteria on beech and oak: the effects of lepidopterous larvae. *Microbiological Research*, *158*(4), 291–7.

Myers, J. H. (2000). Population fluctuations of the western tent caterpillar in southwestern British Columbia. *Population Trends*, *42*, 231–241.

Myers, J. H., & Cory, J. S. (2013). Population Cycles in Forest Lepidoptera Revisited. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 131025103132004.

Myers, J. H., Cory, J. S., Ericsson, J. D., & Tseng, M. L. (2011). The effect of food limitation on immunity factors and disease resistance in the western tent caterpillar. *Oecologia*, *167*(3), 647–55.

Plaistow, S. J., & Benton, T. G. (2009). The influence of context-dependent maternal effects on population dynamics: an experimental test. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1520), 1049–58.

Quesada-Moraga, E., Santos-Quirós, R., Valverde-García, P., & Santiago-Alvarez, C. (2004). Virulence, horizontal transmission, and sublethal reproductive effects of *Metarhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). *Journal of Invertebrate Pathology*, *87*(1), 51–8.

Räsänen, K., & Kruuk, L. E. B. (2007). Maternal effects and evolution at ecological time-scales. *Functional Ecology*, *21*(3), 408–421.

Redford, A. J., & Fierer, N. (2009). Bacterial succession on the leaf surface: a novel system for studying successional dynamics. *Microbial Ecology*, *58*(1), 189–198.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. The Journal of Animal Ecology, 79(2), 403–13.

Saastamoinen, M., Hirai, N., & van Nouhuys, S. (2013). Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. *Oecologia*, *171*(1), 93–104.

Sarfraz, R. M., Cory, J. S., & Myers, J. H. (2013). Life-history consequences and disease resistance of western tent caterpillars in response to localised, herbivore-induced changes in alder leaf quality. *Ecological Entomology*, *38*(1), 61–67.

Shikano, I., Halpert-Scanderbeg, O., Oak, M., & Cory, J. S. (2014). Trade-offs between trans-generational transfer of nutritional stress tolerance and immune priming. Manuscript submitted for publication.

Tammaru, T., & Haukioja, E. (1996). Capital breeders and income breeders among Lepidoptera - consequences to population dynamics. *Oikos*, *77*(3), 561–564.

Tammaru, T., Ruohoma, K., & Gotthard, K. (2004). Compensatory responses in lepidopteran larvae : a test of growth rate maximisation. *Oikos*, *107*, 352–362.

Thorne, C. M., Otvos, I. S., Conder, N., & Levin, D. B. (2007). Development and evaluation of methods to detect nucleopolyhedroviruses in larvae of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). *Applied and Environmental Microbiology*, *73*(4), 1101–6.

Trauer, U., & Hilker, M. (2013). Parental legacy in insects: variation of transgenerational immune priming during offspring development. *PloS One*, *8*(5), e63392.

Triggs, A. M., & Knell, R. J. (2012). Parental diet has strong transgenerational effects on offspring immunity. *Functional Ecology*, *26*(6), 1409–1417.

Vizoso, D. B., & Ebert, D. (2004). Within-host dynamics of a microsporidium with horizontal and vertical transmission : *Octosporea bayeri* in *Daphnia magna*. *Parasitology*, *128*, 31–38.

Xiang, H., Zhu, J., Chen, Q., Dai, F., Li, X., Li, M., Wang, J. (2010). Single base-resolution methylome of the silkworm reveals a sparse epigenomic map. Nature Biotechnology, 28(5), 516–20.

Appendix A.

Development time within food limited parents

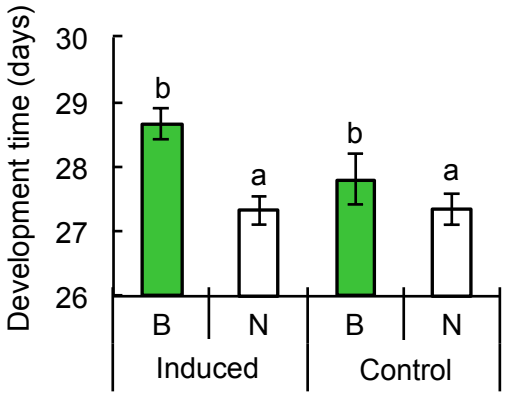


Figure A1. Development time (4th instar - pupation) for food limited *M. c. pluviale* parents (n = 316). Estimates are given as LS Means (± 1 SE). "Induced" refers to a diet of foliage from larvae-infested branches, while "Control" denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application). Lowercase letters indicate significant main effects from application of foliar bacteria; shared letters indicate no difference between treatments.

Table A1. Final minimal statistical models for development time (4th instar - pupation) for food limited *M. c. pluviale* parents (n = 316). P values in bold represent statistically significant (P < 0.05) and marginally non-significant effects. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	*F*	P
Induction	1	1.293	0.256
Microbiota	1	16.653	**<0.001**
Sex	1	30.082	**<0.001**
Initial mass	1	23.938	**<0.001**
Error	311		

Deleted terms: Induction*microbiota: $F_{1,310}$=2.664, P=0.104; induction*sex: $F_{1,309}$=0.891, P=0.346; microbiota*sex: $F_{1,308}$=0.349, P=0.555; induction*microbiota*sex: $F_{1,307}$=1.537, P=0.216.

Appendix B.

Background mortality of virus challenged ad libitum offspring

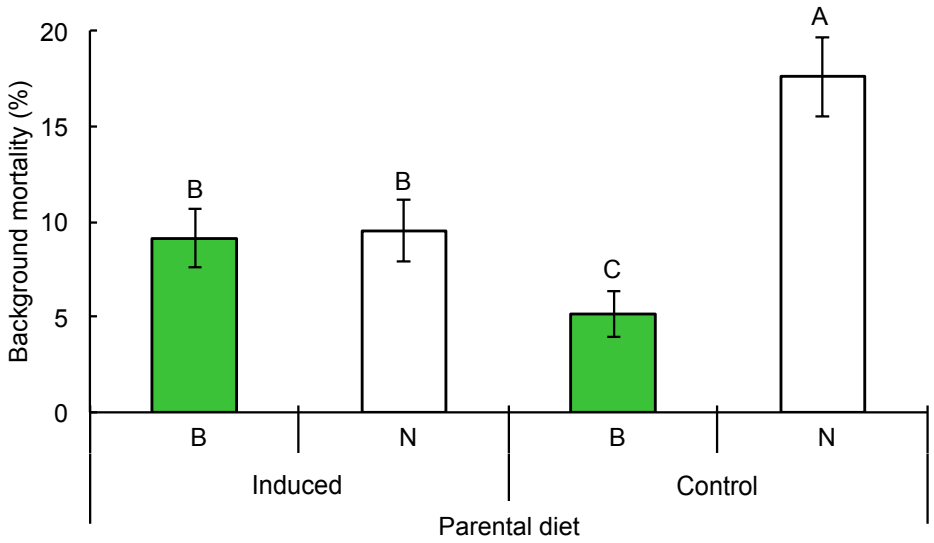


Figure A2. Background mortality within McpINPV challenged larvae for *M. c. pluviale* offspring of ad libitum parents (n = 144). Estimates are based on raw means (± 1 SE). Uppercase letters above bars represent a statistically significant interaction between treatment means (Tukey's test at $P<0.05$). Levels with shared letters are not statistically different. “Induced” refers to a diet of foliage from larvae-infested branches, while “Control” denotes a diet of foliage from branches not under caterpillar attack. B = bacterial application; N = naïve (without bacterial application).

Table A2. Final statistical model for background mortality within McplNPV challenged for larvae for *M. c. pluviale* offspring of ad libitum parents. Statistics were reported from a GLM (binomial, logit). P values in bold represent statistically significant effects at P < 0.05. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

Source	d.f.	$\chi 2$	P
Induction	1	0.029	0.864
Microbiota	1	14.613	**<0.001**
Induction*microbiota	1	12.789	**<0.001**
Block	1	4.184	**0.041**

Deleted terms: Log_{10} dose: χ^2=0.994, P=0.319; initial mass: χ^2=0.015, P=0.901; microbiota*log_{10} dose: χ^2=1.573, P=0.210; induction*log_{10} dose: χ^2=0.529, P=0.467; induction*microbiota*log_{10} dose: χ^2=0.106, P=0.745.

Appendix C

Sublethal effects of virus on offspring pupal mass

Table A3. Final statistical model for pupal mass of offspring given control treatment and virus dosed as a function of parental food quantity for a) females and b) males.

a) Females

Source	d.f.	*F*	P
Quantity	1	2.996	0.092
Error	35.26		

Deleted terms: Initial mass: $F_{1,226.4}$=1.901, P=0.1694; control/dosed: $F_{1,209.9}$=0.690, P=0.407; quantity*control/dosed: $F_{1,203.4}$=1.287, P=0.258.

b) Males

Source	d.f.	*F*	P
Quantity	1	0.821	0.376
Error	19		

Deleted terms: Initial mass: $F_{1,175.5}$=1.479, P=0.226; control/dosed: $F_{1,166.5}$=0.014, P=0.909; quantity*control/dosed: $F_{1,156.7}$=0.544, P=0.462.

Table A4. Final statistical model for pupal mass of ad libitum offspring given control treatment and virus dosed as a function of parental plant induction and microbiota for a) females and b) males. P values in bold represent statistically significant effects at P<0.05. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

a) Females

Source	d.f.	d.f. den	*F*	P
Induction	1	64.49	2.13	0.149
Microbiota	1	65.13	< 0.001	0.988
Block	1	372.6	5.415	**0.021**

Deleted terms: control/dosed: $F_{1,380.2}$=2.537, P=0.112; initial mass: $F_{1,372.2}$=0.365, P=0.546; microbiota*control/dosed: $F_{1,381.2}$=1.945, P=0.164; induction*microbiota: $F_{1,65.37}$=1.535, P=0.220; induction*control/dosed: $F_{1,380.9}$=0.143, P=0.706; induction*microbiota*control/dosed: $F_{1,382.7}$=1.920, P=0.167.

b) Males

Source	d.f.	d.f. den	*F*	P
Induction	1	52.93	0.2728	0.6036
Microbiota	1	51.97	1.1128	0.2964

Deleted terms: Initial mass: $F_{1,272.8}$=1.480, P=0.225; block: $F_{1,256.6}$=0.034, P=0.854; control/dosed: $F_{1,258}$=0.023, P=0.881; induction*microbiota: $F_{1,50.75}$=2.765, P=0.103; microbiota*control/dosed: $F_{1,252.2}$=0.123, P=0.726; induction*control/dosed: $F_{1,249.6}$=0.036, P=0.849; induction*microbiota*control/dosed: $F_{1,250.9}$ =0.977, P=0.324./

Table A5. Final statistical model for pupal mass of ad libitum offspring across virus doses as a function of of parental plant induction and microbiota for a) females and b) males. "Induction" refers to whether leaves came from branches infested with larvae or were protected from herbivory; "Microbiota" refers to whether foliage was dipped in a bacterial suspension or water (naïve).

a) Females

Source	d.f.	d.f. den	*F*	P
Induction	1	62.59	2.2027	0.1428
Microbiota	1	62.69	0.3385	0.5628

Deleted terms: Block: $F_{1,280}$=1.242, P=0.266; initial mass: $F_{1,283.1}$=0.065, P=0.800; $\log_{10}$ dose: $F_{1,276.6}$=0.002, P=0.965; induction*microbiota: $F_{1,63.01}$=2.465, P=0.121; microbiota*$\log_{10}$ dose: $F_{1,273.4}$=0.538, P=0.464; induction*$\log_{10}$ dose: $F_{1,274.9}$=0.026, P=0.872 ; induction*microbiota*$\log_{10}$ dose: $F_{1,608}$=0.168, P=0.682.

b) Males

Source	d.f.	d.f. den	*F*	P
Induction	1	54.79	0.0599	0.8076
Microbiota	1	53.29	0.5047	0.4806

Deleted terms: Log_{10} dose: $F_{1,189.9}$=0.767, P=0.382; block: $F_{1,199.3}$=0.095, P=0.759; initial mass: $F_{1,215.9}$<0.001, P=0.991; induction*$\log_{10}$ dose: $F_{1,191.6}$=1.083, P=0.300; induction*microbiota: $F_{1,52.57}$=0.834, P=0.365; microbiota*$\log_{10}$ dose: $F_{1,187.2}$=0.481, P=0.489 ; induction*microbiota*$\log_{10}$ dose: $F_{1,192.2}$=3.10, P=0.080.