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Viral exposure effects on life-history, flight-related traits, and wing melanisation in the Glanville fritillary butterfly

Luisa Woestmann[a], Melanie Gibbs[b], Helen Hesketh[b], Marjo Saastamoinen[a,*]

[a] Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, PO Box 65, Viikinkaari 1, 00014 University of Helsinki, Finland

[b] NERC Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, United Kingdom

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ABSTRACT

Infections represent a constant threat for organisms and can lead to substantial fitness losses. Understanding how individuals, especially from natural populations, respond towards infections is thus of great importance. Little is known about immunity in the Glanville fritillary butterfly (*Melitaea cinxia*). As the larvae live gregariously in family groups, vertical and horizontal transmission of infections could have tremendous effects on individuals and consequently impact population dynamics in nature. We used the *Alphabaculovirus* type strain *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and demonstrated that positive concentration-dependent baculovirus exposure leads to prolonged developmental time and decreased survival during larval and pupal development, with no sex specific differences. Viral exposure did not influence relative thorax mass or wing morphometric traits often related to flight ability, yet melanisation of the wings increased with viral exposure, potentially influencing disease resistance or flight capacity via thermal regulation. Further research is needed to explore effects under sub-optimal conditions, determine effects on fitness-related traits, and investigate a potential adaptive response of increased melanisation in the wings due to baculovirus exposure.

1. Introduction

The immune system is a crucial component of all organisms, as it defends a host against infections. Such effects are widespread and can cause substantial fitness losses and damage to hosts. Immune defence has evolved in an ecological context, being under strong selection pressure due to ongoing host-parasite co-evolution in an evolutionary “arms race” (Decaestecker et al., 2007; Mone et al., 2010). With increasing global changes due to climate warming and spread of invasive species, alterations in not only prevalence but also severity of some infectious diseases have been suggested to increase significantly (Jones et al., 2008; Shikano and Cory, 2015; Roy et al., 2017), and thus the understanding of how infections impact performance of individuals, especially in wild populations, is of crucial importance. Herbivorous insects are a relevant group for ecological immunological studies, as they are often very sensitive to their environment, and thus might be greatly impacted by any changes in disease dynamics. In the last two decades, eco-immunological studies have expanded also outside model systems, and with increased availability of genomic tools and techniques, our understanding of the impact of biotic and abiotic factors on variation in immunity has greatly improved (Schulenburg et al., 2009; Adamo & Lovett, 2011).

Infection by pathogens often impacts a wide range of host phenotypic traits, such as physiology, morphology, and behaviour (e.g. Poulin & Thomas, 1999; Hoover et al., 2011). Life-history theory suggests that investment in pathogen defence and resistance, however, comes at a cost of other physiological processes, important for individual quality, such as growth and reproduction (Stearns, 1992; Roff, 2002). Fluctuating asymmetry (FA; the stochastic differences between right and left halves of bilaterally symmetrical organisms; Palmer and Strobeck, 1986) has been suggested to represent one measure of developmental instability, and good quality individuals are predicted to show less FA as their genome is able to buffer against environmental influences (Møller & Swaddle, 1997). In many species females choose males based on sexual secondary characters, like courtship, symmetry or wing patterns, that generally reflect individual’s disease resistance (Rantala et al., 2000; Rantala & Kortet, 2003). Pathogens, such as deformed wing virus and nematodes, can provoke drastic effects on morphology in bees and midges (*Chironomus*), respectively (Wülker, 1985; de Miranda & Genersch, 2010). Studies on Monarch butterflies have also shown that pathogenic infection can directly reduce flight ability, however this was not shown to be related to changes in wing morphological traits per se

* Corresponding author.
E-mail addresses: luisa.woestmann@helsinki.fi (L. Woestmann), mela1@ceh.ac.uk (M. Gibbs), hhesketh@ceh.ac.uk (H. Hesketh), marjo.saastamoinen@helsinki.fi (M. Saastamoinen).

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(Bradley & Altizer, 2005). Similarly, in several insects, including Lepidoptera, stressful conditions during development have been shown to impact flight-related traits including wing-morphology (Gibbs et al., 2010; Saastamoinen & Rantala, 2013). In general, the observed responses are often pathological side (or stress) effects but in some cases they have also been shown to represent adaptations of the host or the pathogen (Dingemanse et al., 2009), which are often not investigated and hard to demonstrate (Hughes et al., 2012; van Houte et al., 2013). The responses further depend on the pathogen in question, and are likely coupled with consistent behaviours tightly intertwined with life-history (Kortet et al., 2010). Assessing individual fitness costs in regards to changes in life-history or morphology due to an upregulated immune defence is important in larger context, as it allows us to understand the role of pathogens in regulating natural populations.

In insects, much of the immunity research has focused on model systems, such as *Drosophila* (Lemaitre & Hoffmann, 2007). In Lepidoptera, moths, on the other hand, have been the main focal system of immune studies, whereas immunity in butterflies is less well understood (e.g. Reeson et al., 1998; Wilson & Graham, 2015, but see e.g. Lindsey & Altizer, 2008). Baculoviruses are DNA viruses that are highly pathogenic and obligate killers of insects and other arthropods. They represent a good model system for exploring viral infections in insects as they predominantly infect lepidopteran species (Cory & Myers, 2003). Baculoviruses also have the potential to influence host population dynamics (Dwyer & Elkinton, 1993) and they are widely used as control agents for insect pests through inundative applications (Cory and Myers, 2003; Lacey et al., 2015). Viral horizontal transmission is achieved via environmentally stable occlusion bodies, which are produced at the end of the infection cycle in host tissues and released into the environment where they can be ingested by new hosts. Horizontal transmission is known to have an important role in pathogen disease progression (e.g. McCallum et al., 2001). With increasing host density, the influence of pathogens on population dynamics is often more dramatic (although simple mass action models are influenced by many factors including spatial structure, pathogen clumping and behaviour (e.g. d'Amico et al., 2005).

We used a species from the *Alphabaculovirus* genus of baculoviridae, specifically the virus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), to investigate the effects of viral exposure on survival, developmental life-history, flight-related traits, wing melanisation and wing symmetry in the Glanville fritillary butterfly (*Melitaea cinxia*). Even though viral infections are likely to be important in natural populations of this species, little is known about the full range of pathogens the Glanville fritillary encounters in nature. This study is hence the first to experimentally test the response of this butterfly to an exemplar viral infection. Moreover, as effects of a baculovirus infection in the larval stage on adult traits are not widely investigated so far, our study also provides new information more generally. Host-pathogen interactions are likely to be relevant in this species due to its life-history: females lay eggs in clusters and the larvae live gregariously throughout most of their development. This means that horizontal transmission of pathogens may be particularly effective, as parasite transmission is usually positively density dependent (Anderson & May, 1981; McCallum et al., 2001, but see Wilson et al., 2003). Furthermore, as in Finland the Glanville fritillary persists as a classical metapopulation in the Åland islands, dispersal represents a key life-history trait. Any potential effects of viral infection on flight-related traits that influence adult flight ability could therefore have important fitness consequences for this species. The specific aims of this study were to 1) determine the viral concentration-mortality relationship in sixth instar larvae of the Glanville fritillary butterfly exposed to AcMNPV in the laboratory, 2) assess virus concentration-dependent effects on larval developmental traits and 3) investigate whether adults show changes in flight-related wing morphology or body allocation related to viral exposures during development that could potentially influence their flight ability. We hypothesized that physiological constraints caused by resources being allocated to immune defence during growth would result in resource allocation trade-offs across life stages with fewer resources available to invest in life-history traits, such as developmental time or daily mass acquisition within the larval stage, and/or fewer resources available to invest in wing morphological traits in the adult stage. Little is known about the effects of baculovirus infection on wing development and wing morphological traits, but previous studies on another butterfly species found that exposure to viral infection during development affects resource allocation to thorax mass (Hesketh et al., 2012; Gibbs & Weir, 2017). We therefore predicted that effects of baculovirus infection on wing development (i.e. wing symmetry) and flight-related wing morphology (i.e. forewing length, forewing area, loading, forewing aspect ratio, thorax ratio) may also be apparent in the Glanville fritillary. In addition, we hypothesized that due to constraints, increased need of melanin for immune defence would result in reduced melanisation of adult wings. Finally, as immune response is thought to vary generally based on different life-histories for males and females (Rolff, 2002), we expected to observe sex-dependent differences in response to viral exposure in the Glanville fritillary.

2. Material and methods

2.1. Study species

In Finland, the Glanville fritillary butterfly, *Melitaea cinxia* (Melitaeini: Nymphalidae), is present only in the Åland islands in the south-western edge of the mainland. The habitat of the butterfly is highly fragmented in the Åland islands, and the butterfly persists there in a classic metapopulation. The metapopulation consists of a network of around 4000 habitat patches of dry meadows within an area of 50 × 70 km (Hanski, 1999; Nieminen et al., 2004). Larvae utilize two plants as their food source, *Plantago lanceolata* and *Veronica spicata*. Having a univoltine life-cycle, larvae feed during the first five instars on the host plant and overwinter in a silken web during diapause. They continue feeding in spring, moult into two to three more instars and pupate in May followed by adults in June to mid-July, which feed on nectar. Females emerge about 2–3 days later than males and carry the full number of oocytes in their ovarioles, which they lay in several clutches on the larval host plants (Boggs & Nieminen, 2004). Females show higher dispersal rates than males (Kuussaari et al., 1996) and the longest colonization distances recorded are about 4–5 km (Van Nouhuys & Hanski, 2002). Males, which typically perform short flight bouts and rapid take-offs establishing or defending their mating territory, are, however, also able to fly more continuously in search for females (Boggs & Nieminen, 2004).

2.2. Baculovirus production

A stock suspension of the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was produced as described previously in Gibbs et al. (2010) and Gibbs and Weir (2017). To enumerate the concentration of the stock, occlusion bodies (OBs) were counted in an improved Neubauer haemocytometer at magnification 400× (< 10% error in counts) and this was replicated three times to give an estimated average concentration of OBs ml^{-1}. The stock suspension was stored at −20 °C until required. Serial dilutions were prepared in sterile distilled water from the stock suspension for experiments to give seven different final concentrations ranging between 1×10^3 and 1×10^9 OBs ml^{-1} of 10-fold differences at each concentration.

2.3. Experimental design

2.3.1. Experiment 1: effects of increasing concentration exposure to AcMNPV

This experiment was designed to examine the impact of exposure of sixth instar larvae to increasing concentrations of AcMNPV on larval