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Altered nutrient intake by baculovirus-challenged insects: Self-medication or compensatory feeding?

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ABSTRACT

Infection by parasites can alter the feeding behaviour of hosts. Some animals seek out substances that can therapeutically clear infections (self-medication), some may seek out resources to recoup resources lost while fighting off infection (compensatory feeding) and others may be manipulated to ingest substances that benefit parasite fitness (parasite manipulation of host). Recent studies have indicated that pathogen-challenged insects can self-medicate by increasing their protein intake relative to carbohydrate, which is thought to act by boosting the insect's immune response. However, increased protein intake could also be due to compensatory feeding or pathogen manipulation of the host, and a rigorous examination of all four of the testable predictions, which is necessary for verifying self-medication behaviour, has not been conducted. The therapeutic behaviour must (1) only be employed by infected individuals and (2) alleviate the potential fitness loss of the infected individual. (3) If an uninfected individual engages in the behaviour, they suffer a decrease in fitness, and lastly, (4) the parasite cannot benefit from the behaviour. In response to baculovirus-challenge (AcMNPV) at 24 °C, the cabbage looper, *Trichoplusia ni*, increased proportional protein intake, by increasing protein intake rather than decreasing carbohydrate intake. Increased protein intake did not benefit virus fitness, but it also did not increase the probability of host survival. Increased proportional protein intake did not occur in response to TnSNPV-challenge at 24 °C or in response to AcMNPV-challenge at a higher temperature (32 °C), indicating that the virus-induced change in nutrient intake depends on virus identity and temperature. Since virus-challenged *T. ni* did not show the typical costs associated with infection, the altered nutrient intake is likely to be a compensatory response. Understanding the motivation behind pathogen-induced changes in feeding behaviour could have significant implications for determining its importance for species interactions at multiple trophic levels.

1. Introduction

Animals face a continuous onslaught from a wide range of parasite species that can inflict severe fitness costs, including retarded growth, reduced reproduction and death. Accordingly, animals have evolved a variety of defense strategies to avoid or prevent infection and constrain parasite growth. All animals have physiological defenses against parasites. The host's physical barriers, such as the skin and intestinal mucin, prevent entry of most parasites, and parasites that circumvent these barriers are most often destroyed and cleared by the host's immune system. Some animals can also engage in behavioural defense, such as the avoidance of infected conspecifics (Behringer et al., 2006) and contaminated foods (Hutchings et al., 2001; Parker et al., 2010). Some animals can contact toxic or non-toxic substances prophylactically in response to parasite risk (Chapuisat et al., 2007) (including transgenerational prophylaxis in flies and monarch butterflies; (Kacsoh et al., 2013; Lefèvre et al., 2012, 2010)) and therapeutically in response to infection (Milan et al., 2012; Singer et al., 2009).

Although only higher vertebrates with the capacity to observe and learn were believed to be able to medicate themselves after infection, the discovery of therapeutic medication (also termed self-medication) in insects, revealed that the behaviour can also be innate (de Roode et al., 2013). Singer et al. (2009) proposed that self-medication is a type of adaptive plasticity, and defined it as a parasitism- or disease-induced change in behaviour or phenotype during an individual's lifetime that improves its probability to survive and reproduce. To demonstrate that the behaviour is truly an adaptation that allows infected hosts to fight their parasites, it must meet four essential criteria. (1) Only infected individuals

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engage in the therapeutic behaviour, and this (2) must alleviate the potential fitness loss of the infected individual (Clayton and Wolfe, 1993). (3) If an uninfected individual engages in the behaviour, they suffer a decrease in fitness (Clayton and Wolfe, 1993; Singer et al., 2009). In some instances, altered host behaviour that increases survival can also benefit the parasite by increasing parasite reproductive output (Karban and English-Loeb, 1997). Therefore, the final criterion is that (4) the parasite cannot benefit from the behaviour (Lefèvre et al., 2010, 2009). Self-medication behaviours meeting these criteria have been demonstrated in insects that use secondary metabolites as a medicating substance. Woolly bear caterpillars, *Grammia incorrupta*, parasitized by parasitoid flies increased consumption of secondary plant metabolites, pyrrolizidine alkaloids, which are toxic to both the host and the developing parasitoid (Singer et al., 2009; Smilanich et al., 2011). Fruit flies, *Drosophila melanogaster*, parasitized by parasitoid wasps increased their consumption of ethanol, a secondary metabolite produced by yeasts growing on rotting fruit (Milan et al., 2012).

Insect self-medication is suggested to extend beyond the use of secondary metabolites to include the altered intake of macronutrients. Caterpillars of two noctuid moth species, *Spodoptera littoralis* and *Spodoptera exempta*, selectively increased their intake of protein relative to carbohydrate (or higher *p:c* ratio) after ingesting a moderate dose of a lethal baculovirus (Lee et al., 2006; Povey et al., 2013). *S. exempta* also selectively increased its dietary *p:c* ratio after intra-haemocoelic injection of the bacterium, *Bacillus subtilis* (Povey et al., 2009). While the first study (Lee et al., 2006) concluded that the altered feeding behaviour was a compensatory response to offset the nitrogen (or protein) resources used to fight infection, the two more recent studies (Povey et al., 2013, 2009) and a review paper (Abbott, 2014) have categorised this behaviour as self-medication. In these studies, pathogen-challenged individuals selectively increased their proportional protein intake while unchallenged individuals did not (criterion 1), and those that survived infection selected a higher *p:c* ratio diet than those that died. This behaviour coincided with higher survival of pathogen-challenged hosts that were restricted to higher *p:c* ratio diets (criterion 2). Like many substances, the ingested dose largely determines whether protein will act as a nutrient, medicine or toxin. Dietary restriction to excessively protein-biased diets is well-known to be costly (criterion 3), resulting in slower development rate and shortened lifespan (e.g. Lee et al., 2002, 2006, 2008; Shikano and Cory, 2014a). Lastly, since the pathogens (baculoviruses and many other entomopathogens) require host death to release infectious transmission stages, increased host survival is assumed to directly reduce pathogen fitness, at least at the population level. However, among hosts that engage in the behaviour, some will still succumb to infection. None of the three studies investigated whether the pathogens may have benefited in these killed hosts (criterion 4).

Our objective in this study was to decipher whether the altered nutrient intake of baculovirus-challenged caterpillars is truly self-medication behaviour or if it might be compensatory feeding or host manipulation by the virus. We challenged the cabbage looper, *Trichoplusia ni*, larvae with one of two species of baculovirus, *T. ni* singly-enveloped nucleopolyhedrovirus (TnSNPV) and *Autographa californica* multiply-enveloped NPV (AcMNPV), and tested whether any virus-induced change in host nutrient intake met all of the criteria of self-medication. We have previously shown that *T. ni* survival after challenge with TnSNPV or AcMNPV increases with the proportion of protein in the diet (Shikano and Cory, 2015). Thus, we first examined whether increased proportional protein intake occurs in virus-challenged larvae (test of criterion 1) and estimated how much this behaviour would increase the probability of survival, based on mortality data from our previous study (test of criterion 2). We also assessed whether a higher self-selected intake of protein is detrimental to naïve *T. ni* and survivors of virus-challenge by evaluating the association of proportional protein intake with pupal weight and development time (test of criterion 3). Lastly, we examined if a higher self-selected proportional protein intake by virus-infected *T. ni* was beneficial for virus fitness by evaluating the association between proportional protein intake and weight of virus-killed cadavers (an estimate of the number of infectious transmission stages produced; Wilson et al., 2000) and speed of kill (test of criterion 4). Since, the performance of AcMNPV-challenged larvae, which is a composite measure including survival, pupal weight and development time, improved more with increasing *p:c* ratio at higher temperatures (Shikano and Cory, 2015), we investigated the potential impact of temperature on nutrient-based self-medication behaviour at two temperatures (24 and 32 °C) for experiments involving AcMNPV.

2. Materials and methods

2.1. Insect and virus

Cabbage looper, *T. ni*, eggs were obtained from a laboratory colony, which was initiated from larvae collected from a commercial tomato greenhouse in British Columbia, Canada (Janmaat and Myers, 2005). They have been maintained at 25 °C and 16L:8D photoperiod on a wheat germ-based artificial diet, which has a protein to carbohydrate ratio of approximately 1p:1.1c (Bio-Serv, Frenchtown, NJ, USA). *T. ni* from this colony tightly regulate their nutrient intake (Shikano and Cory, 2014a) and select a nutrient intake target that is near-optimal for development (Shikano and Cory, 2014b). Larvae that had been reared individually from the neonate stage until the slippage of the head capsule in the late fourth instar were used in the experiments. Isolates of TnSNPV (isolate FV#3433) and AcMNPV (strain E2) were both amplified in *T. ni*. The concentration of viral occlusion bodies (OBs) was estimated using an improved Neubauer brightline haemocytometer (0.1 mm deep; Hausser Scientific) at 400× magnification on a phase contrast microscope. Both viruses are found in wild *T. ni* populations (Jaques, 1970; Theilmann et al., 2005). TnSNPV is only known to infect *T. ni* (i.e. specialist) (Del Rincón-Castro and Ibarra, 1997) while AcMNPV has a broad host range, infecting at least 15 lepidopteran families (i.e. generalist) (Cory and Myers, 2003).

2.2. Administration of viruses

Newly moulted, unfed final (fifth) instar *T. ni* larvae were weighed to the nearest 0.1 mg and challenged with 360 OBs of TnSNPV or AcMNPV. This dose was previously shown to kill 20–68% of TnSNPV-challenged and 30–60% of AcMNPV-challenged *T. ni*, depending on temperature and dietary protein to carbohydrate ratio consumed after virus-challenge (Shikano and Cory, 2015). At the optimal *p:c* ratio for *T. ni*, the mortality produced by this dose of TnSNPV or AcMNPV (33 and 39% respectively) was not statistically different (Shikano and Cory, 2015). Virus OBs suspended in 2 μl of distilled water were applied to a 3 × 2 mm (length × diameter) diet plug, made from the wheat germ-based artificial diet used to maintain the colony insects. Distilled water was applied to diet plugs to serve as a control. For the TnSNPV experiment, 140 larvae were individually provided with an OB-treated diet plug and 70 larvae were provided with a control diet plug. Larvae fed on their diet plug in individual wells of a 24-well cell culture plate for 24 h. All larvae consumed their entire diet plug and were transferred to individual 30 ml plastic cups to choose their intake of dietary protein and carbohydrate (see below). Insects were maintained at 24 °C and 16L:8D photoperiod. Although giving larvae 24 h to consume the diet plug is common practice, it was evident from this experiment that all