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Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth (Galleria mellonella)

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Abstract  The resources available to an individual in any given environment are finite, and variation in life history traits reflect differential allocation of these resources to competing life functions. Nutritional quality of food is of particular importance in these life history decisions. In this study we tested trade-offs among growth, immunity and survival in three groups of greater wax moth (*Galleria mellonella*) larvae fed on diets of high and average nutritional quality. We found rapid growth and weak immunity (as measured by encapsulation response) in the larvae of the high-energy food group. It took longer to develop on food of average nutritional quality. However, encapsulation response was stronger in this group. The larvae grew longer in the low-energy food group, and had the strongest encapsulation response. We observed the highest survival rates in larvae of the low-energy food group, while the highest mortality rates were observed in the high-energy food group. A significant negative correlation between body mass and the strength of encapsulation response was found only in the high-energy food group revealing significant competition between growth and immunity only at the highest rates of growth. The results of this study help to establish relationships between types of food, its nutritional value and life history traits of *G. mellonella* larvae.

Key words *Galleria mellonella*, growth, immunity, life-history, nutrition, survival
Introduction

According to life history theory organisms attempt to allocate limited resources to primary life functions related to growth, reproduction, and self-maintenance in ways that optimize reproductive fitness (Stearns, 1992; Charnov, 1993; Roff, 2002). Susceptibility to disease is ubiquitous because of trade-offs between immunity and other needs of the organism (Schmid-Hempel, 2011). These trade-offs often cause considerable self-harm to the immune system, and most organisms do not grow at their maximal rate (Arendt, 1997; Mangel & Stamps, 2001; Roff et al., 2004; Fedorka et al., 2004; Alonso-Alvarez et al., 2007; Bascuñán-García et al., 2010).

In unpredictable environments, the ability to grow and reproduce as fast as possible is of crucial importance. The early reproduction and acquisition of a large body size can incur benefits such as earlier reproductive output and reduced risk of predation (Metcalfe & Monaghan, 2003). However, there are also many costs of growing fast. Individuals with rapid growth might be more exposed to predators because of more time spent foraging (Sorci et al., 1996; Munch & Conover, 2003). They may be more susceptible to starvation during periods of food shortage (Arendt, 1997; Blanckenhorn, 2000), and their growth rates might interfere with development (e.g. Fisher et al., 2006; Pihlaja et al., 2006) because the increase in metabolic activity needed to fuel rapid growth could cause oxidative damage to the organism (e.g. Farrell et al., 1997; Morgan et al., 2000; Forsén et al., 2004). It has also been shown that the nutritional state of the host, and nutritional quality of food, may have a profound effect on life-history trade-offs and ability to fight and resist an infection (Alonso-Alvarez & Tella, 2001; Moret & Schmid-Hempel, 2000; Siva-Jothy & Thompson, 2002; Ayres & Schneider, 2009; Cotter et al., 2011; Ponton et al., 2011, 2013; Jiménez-Cortés et al., 2012; Jiménez-Cortés & Córdoba-Aguilar, 2013; Povey et al., 2013).
A highly elaborate immune system is required by organisms with long life expectancy, those requiring extensive parental care until they mature, and those living in predictable environments. However, while immunity has the obvious potential to ameliorate infection outcomes, immune responses require increased metabolism (Freitak et al., 2003; Krams et al., 2014) and immunity can also harm hosts by either damaging host tissues or monopolizing resources leading to increased mortality (Kraaijeveld & Godfray, 1997; Fellowes & Godfray, 2000; Kraaijeveld et al., 2001; Jensen et al., 2006; Sadd & Siva-Jothy, 2006; Little & Killick, 2007). The costs of immunity are either associated with genetic differences among hosts where some genotypes invest heavily in defense systems at the expense of other functions, or are associated with the cost of launching an immune response (Schmid-Hempel, 2011).

The greater wax moth (Galleria mellonella) is a moth of the family Pyralidae which is found in most of the world. This moth flies from May to October in the temperate parts of its range in the Northern hemisphere. The larvae feed on the honeycomb inside bee nests and may become important pests of apiculture (Warren & Huddleston, 1962). However, the presence of adult bees prevents wax moth damage, and destruction to combs usually occurs within weak hives with low populations. It has been suggested that when a colony of honey bees dies from one or more diseases in the wild (Barjac & Thomson, 1970) the wax moth will quickly clean up the natural holes for new bee colonies to breed. This indicates that the first generation of the greater wax moth to invade a bee colony usually has larger amounts of food resources than any further generation. The whole lifecycle of the greater wax moth, and extent of population expansion, depends on a suitable temperature range and adequate food. Observations show that the larvae are highly resistant to food shortage, but under deficient food conditions, their development (from egg to adult) may be extended up to 6 months. Adult insects from poorly nourished larvae are smaller and their survival is decreased.
(Marston et al., 1975). In the absence of adequate food supplies the larvae become cannibalistic.

Under conditions of foods of low nutritional value, individual investment should change from growth to immune function because longer lifespan needs elaborated immune system. In this study we provided one group of larvae of the greater wax moth with high-energy food *ad libitum* during their development, one group with food of average quality, and another with food resources containing low energy. We assessed immunity via encapsulation response to nylon monofilament implantation (e.g. Rantala et al., 2000, 2002; Kivleniece et al., 2010). We predicted the most rapid growth and weakest immunity in the high-energy food group. In the low-energy food group we predicted slower growth and stronger immunity. After activation of the immune system we expected higher mortality and less successful pupation in the high-energy food group. We did not expect any intermediate investment in the immune system because the average levels of immune protection are not likely linked to benefits of longer lifespan (Schmid-Hempel, 2011).

**Materials and Methods**

**Insects**

We studied a captive population of *G. mellonella* consisting of individuals originated from the stock of the University of Turku mixed with individuals collected from natural populations in Estonia. Our stock culture was maintained at the University of Daugavpils. Moths were reared in 2.4 L plastic boxes at 28–30°C. The larvae used in this study were obtained from 100 males and 100 females.
Food quality and experimental trials

To study the effect of food quality on the strength of encapsulation response, the body size at pupation, and survival of *G. mellonella* larvae, we kept the larvae on food of high nutritional value between hatching and day 15 post-hatch. On day 15 we assigned them to three groups differing in nutritional value (Fig. 1). Each larva was placed individually into plastic containers with a lid and wire-mesh to allow ventilation and to prevent individuals from escaping. Body weight of larvae was similar across the three groups on day 15 post-hatch (one-way ANOVA: $F_{2,107} = 1.72, P = 0.19$).

The high-energy food group received only food of the highest nutritional value provided *ad libitum* until day 30 (Fig. 1) when 10% of larvae initiated pupation. The average-energy food group received food of high nutritional quality provided *ad libitum* for 2 days followed by another 2 days on low quality food and this combination of foods was provided until day 30 post-hatch. The low-energy food group received only *ad libitum* food of low quality and nutritional value from hatching till day 30 post-hatch. The high-energy food consisted of a mix of the same parts of honey, glycerol, bee-wax, dried milk, wheat flour, dry yeast, distillate water and two parts of corn meal. The amount of food energy associated with this food was estimated as ca.16.90 kJ/g. The low-energy food consisted of natural bee wax only. Bee-wax is a natural polymer produced by bees, and it is considered to have an extremely low nutritional value. However, we observed the ability of some wild progenitors of our study population to reproduce solely on bee-wax.

On day 30 post-hatch we weighed all of the larvae and assigned individuals of all three groups to two subgroups: survival subgroup and treatment subgroup. We recorded the time of pupation in the survival subgroup, while in the treatment subgroup, the larvae...
received one sterile nylon monofilament implant (2 mm length, 0.18 mm diameter, knotted at one end) through their cuticle between the third and fourth sternite (Krams et al., 2011a,b; Daukšte et al., 2012) for 5 hours at 29 ± 0.5°C. During implantation the larvae were kept in individual small plastic containers containing no food, and, as soon as 5 h were over, we removed the implants (Fig. 1) and placed the larvae back to their individual containers with food. We checked their survival for 72 hours to see survival and pupation rates. This was done to see whether there is any difference among the three groups in their ability to resist costs of the activation of the immune system.

**Immune assays**

The strength of the encapsulation response was operationalized as the lightness of the nylon filament insert after it had been dried. Insect immune systems respond to this challenge as if the insert were a foreign body, by attempting to encapsulate it in a coating of cellular materials and chemical deposits (e.g. Rantala et al., 2000). The stronger the immune response to the insert, the darker the encapsulation (Yourth et al., 2001; Krams et al., 2013a,b), due to phenoloxidase enzyme production activated by the immune response, resulting in melanization of the capsule (Ratcliffe et al., 1985). In other insects, the encapsulation response is correlated with measures of immunity such as the phenoloxidase cascade (Rantala et al., 2000, 2002, 2003a), encapsulation of parasites (Paskewitz & Riehle, 1994; Gorman et al., 1998), and to resistance of an entomopathogenic fungal disease (Rantala & Roff, 2007).

Lightness was assessed from photographs of the inserts taken from two directions under constant light conditions using a Zeiss Lumar V12 Stereo (Carl Zeiss, Jena, Germany) microscope. Digital images were analysed using the Image J software (http://rsbweb.nih.gov/ij/; Abramoff et al., 2004). Prior to this, we calibrated reflectance of an
implant before the insertion to zero level.

Statistical analysis

Developmental time, body mass and the strength of encapsulation response were distributed normally (Kolmogorov–Smirnov test, all \( P > 0.05 \)) and parametric statistics were used in the further analyses. We used one-way ANOVA and post-hoc tests to find any possible differences among the groups. Multiple linear regression analysis was used to find out determinants of developmental time and the strength of encapsulation response. Analyses were performed in SPSS 17 for Windows (Chicago, SPSS Inc., IL, USA).

Results

Developmental time, body mass, encapsulation and survival

The first larvae of the high-energy food group pupated on day 28 post-hatch (31.30 ± 2.47, mean ± SD). All individuals of high-energy and average-energy groups survived until day 30 when we performed experimental manipulations. In contrast, only 30 out of 50 larvae survived until day 30 post-hatch in the low-energy food group (Fisher’s exact test: \( P = 0.0001 \)) suggesting a role of food quality in larval survival. The time of pupation significantly differed among the groups (one-way ANOVA: \( F_{2,87} = 1765.60, P < 0.0001 \), Fig. 2). The first larvae of the average-energy food group pupated on the day 32 post-hatch (34.63 ± 1.13, mean ± SD), and this was significantly later than the onset of pupation in the high-energy food group (Tukey’s post-hoc test: \( P < 0.001 \), Fig. 2). The larvae of the low-energy group
reached pupation phase on day 59 post-hatch (65.20 ± 3.17, mean ± SD) which was significantly later than the day of pupation observed in the average-energy food group (Tukey’s post-hoc test: \( P < 0.001 \), Fig. 2).

Body mass differed among the larvae on day 30 post-hatch (one-way ANOVA: \( F_{2,87} = 26.40, P < 0.0001 \), Fig. 3), and it was significantly greater in the high-energy food group (0.105 ± 0.045 g, mean ± SD) than body mass of individuals kept on average-energy food (0.078 ± 0.017 g, mean ± SD) (Tukey’s post-hoc test: \( P = 0.001 \), Fig. 3) and individuals of the low-energy food group (0.053 ± 0.011 g, mean ± SD) (Tukey’s post-hoc test: \( P < 0.0001 \)), while individuals in the average-energy food group were heavier than the larvae of low-energy food group (Tukey’s post-hoc test: \( P = 0.003 \), Fig. 3).

The encapsulation response against the nylon monofilament differed significantly among the three food groups (one-way ANOVA: \( F_{2,87} = 62.32, P < 0.0001 \); Fig. 4) on the day of pupation of the larvae of high-energy food group. The strength of encapsulation response of larvae from the high-energy food group (13.06 ± 14.75, mean ± SD) was significantly weaker than that of larvae from the average-energy food group (32.88 ± 25.95, mean ± SD) (Tukey’s post-hoc test: \( P < 0.0001 \), Fig. 4), and weaker than encapsulation response of larvae of the low-energy food group (66.27 ± 12.38, mean ± SD) (Tukey’s post-hoc test: \( P < 0.0001 \), Fig. 4). The strength of encapsulation response of larvae from the average-energy food group was lower than encapsulation response of larvae from low-energy food group (Tukey’s post-hoc test: \( P < 0.0001 \), Fig. 4).

**Determinants of pupation time and encapsulation response**

Multiple linear regression analysis (adjusted \( R^2 = 0.57, F_{2,87} = 55.32, P < 0.0001 \)) showed that encapsulation response of the larvae was dependent on the quality of food (\( \beta = -0.75, P \))
< 0.0001), while body weight ($\beta = -0.01, P = 0.87$) was not a significant determinant of encapsulation response, demonstrating that high-energy food decreased the strength of encapsulation response in *G. mellonella*. Entry of the interaction term for food group and body mass into the model did not result in a significant increase in the adjusted $R^2$ (0.57, $P >0.05$) and the interaction did not reach significance in the model ($\beta = 0.37, P = 0.45$) suggesting that food quality was the primary determinant of encapsulation response.

Multiple linear regression analysis also showed (adjusted $R^2 = 0.80, F_{2,87} = 181.02, P < 0.0001$) that developmental time of larvae was not dependent upon the strength of encapsulation response ($\beta = 0.09, P < 0.24$), while it was dependent on food quality ($\beta = -0.83, P < 0.001$), demonstrating that food of low energy-content increased the larval development in *G. mellonella*. Entry of the interaction term for encapsulation response and food type into the model resulted in a significant increase in the adjusted $R^2$ (0.89, $P < 0.0001$) and the interaction reached significance in the model ($\beta = -0.72, P < 0.0001$). We also found that the strength of encapsulation response is significantly negatively correlated with body mass only in the high-energy food group ($r = -0.44, P = 0.015$ vs $r = -0.05, P = 0.78$ in the average-energy group and $r = 0.18, P = 0.35$ in the low-energy food group).

**Survival after activation of immune system**

Fourteen of 30 larvae survived and pupated in the high-energy group over the course of three days upon removal of nylon implants. The larvae survived significantly better (26 out of 30) in the average-energy food group (Fisher’s exact test: $P = 0.002$), and in the low-energy group (28 out of 30) (Fisher’s exact test: $P = 0.0001$) than in the high-energy food group. We did not find any significant difference in survival of larvae in the average-energy and low-energy food groups (Fisher’s exact test: $P = 0.67$).
Discussion

In this study we found rapid growth, earlier pupation and weak encapsulation response in the larvae of the high-energy food group. It took longer to develop in the average-energy group, while encapsulation response was stronger in this group. The larvae grew longer in the low-energy food group, and had the strongest encapsulation response. The highest survival rates were observed in larvae of the low-energy food group, while the highest mortality rates were observed in the high-energy food group. Finally, we found a significant negative correlation between body mass and the strength of encapsulation response only in the high-energy food group.

There is some evidence suggesting that nutrition at early stages of ontogeny may substantially affect such life history traits as developmental time, body size, reproductive success and survival at maturity (Nylin & Gotthard, 1998; Lindstrom, 1999; Metcalfe & Monaghan, 2001; Monaghan, 2008; Dmitriew, 2011). Our study supports previous findings by showing that *G. mellonella* can afford fast larval development and greater body weight at pupation only if food of the highest nutritional quality is available. We show that poor nutrition resulted in decreased body size of *G. mellonella* at pupation, prolonged larval development and high mortality as has previously been shown (Marstone *et al.*, 1975). While we do not have data on the immune function of the larvae that died before the strength of encapsulation response was measured, our results show that longer developmental time is associated with stronger encapsulation response as it was found in the average- and low-energy food groups. Low encapsulation response was a likely explanation for the high mortality rate after activation of the immune system in the larvae of the high-energy food group. Moreover, a significant negative correlation between body mass and the strength of encapsulation response was found only in *G. mellonella* larvae of the high-energy food group.
showing a trade-off between immune function and larval growth.

After hatching, larvae tunnel in comb, lining their tunnels with silky web as they go. Thus, the food provides not only nutrition and cover against bees but also protection against parasites and pathogens. Most of bee products and especially honey have antiseptic, antibiotic, antifungal, and antibacterial properties, which means that larvae of the high-energy food group might be more protected against different kinds of pathogens than larvae in the remaining groups because of a higher content of honey in their food. Therefore, the enhanced anti-bacterial properties of larval environment in the high-energy food group might represent one more reason for their reduced investment in immunity. Future research should disentangle the nutritional and antibacterial effects of food in life history trade-offs of G. mellonella.

It is noteworthy that the amount of food of the highest quality provided ad libitum in the average-energy group was the same as in the high-energy food group between day 1 and day 15 post-hatch, while high-energy nutrition was available only within 50% of the larval development between day 16 and the pupation. In total, it makes ca.75% of time spent on high-energy food in the average-energy food group. Body mass of the larvae prior to pupation was 25% greater in the high-energy food group than in the average-energy food group which explains the effect of food quality. However, encapsulation response of individuals of the average-energy group was twice as strong as that of the high-energy food group. This suggests that fast and slow larval development might represent different strategies with no transitional strategies in between. This idea is supported by the fact that longer larval development needs elaborated immune system while insufficient investment in the immune system may lead to higher mortality as has been observed in terminal investment into reproduction in mealworm beetles (Krams et al., 2011).

Our study design did not allow us to identify sex-specific effects of diet on larval
development, immune status, and body weight at pupation. The most reliable sex
determination in *G. mellonella* can be done at the pupa stage, while many individuals died
already at the larval stage in this study. Since diet was shown to have sex-specific effects on
larval and nymphal development, immune system, sexual attractiveness and reproductive
success at maturity (Kelly & Tawes, 2013; Kelly et al., 2014), research on sex-specific
effects on larval development of *G. mellonella* should be performed using a different study
design in the future. However, our results suggest that the data on the strength of
encapsulation response, developmental time and body mass in each of the groups were
normally distributed. This indicates that sex-related factors may have rather minor role than
the effect of diet on the strategy of larval development. Perhaps this is because both sexes
vigorously fly and male greater wax moths are only slightly smaller than females. In this case
a poor diet at the larval stage would force both males and females to allocate resources to
immune function rather than growth. This might be expected to be opposite in such species as
the vapourer moth (*Orgyia antiqua*) where females are wingless, much larger than males and
in which larger female moths are generally more fecund (Tammaru et al., 2002). Increasing
body size should benefit female fitness and we expect that species with large females would
allocate more resources to growth than to immunity.

Some studies showed that larvae switching from a carbohydrate-biased diet onto a
diet that is relatively protein-rich will generally have more haemocytes and higher levels of
PO with which to melanize and encapsulate virus-infected cells (Washburn et al., 1996;
Trudeau et al., 2001), which contradicts our results. Some other studies also showed that poor
nutrition and stressors such as heavy metal pollution may increase investments in the immune
system (de Block & Stoks, 2005, 2008; van Ooik & Rantala, 2010; Pölkki et al., 2012, but
see Banville et al., 2012). This suggests that the theory linking growth, immunity,
reproduction, parasite- and nutrient-related effects is still in its infancy and more research is
needed to explain the evolution of life-history strategies. Finally, the larvae of the greater wax moth *G. mellonella* are increasingly used as hosts to study pathogenesis and virulence factors of many prominent bacterial and fungal human pathogens, and as a reliable host model to evaluate the efficacy of new kinds of drugs (Vilcinskas, 2011; Vogel *et al*., 2011; Dubovskiy *et al*., 2013a,b) The results of this study will help to establish relationships between types of food, its nutritional value and life history traits of *G. mellonella* larvae to develop standardized food types and make inter-laboratory comparisons possible.

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**Disclosure**

The authors have no any conflict of interest.
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Fig. 1 Time course of the study.
**Fig. 2** The effects of food quality on duration of larval development of *Galleria mellonella*. Bars show means ± SE.
**Fig. 3** The effects of food quality on the larval body mass prior pupation in *Galleria mellonella*. Bars show means ± SE.
Fig. 4 The effects of food quality on the strength of encapsulation response of *Galleria mellonella* larvae. Bars show means ± SE.