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Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection

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ABSTRACT

Parasites are dependent on their hosts for energy to reproduce and can exert a significant nutritional stress on them. Energetic demand placed on the host is especially high in cases where the parasite-host complex is less co-evolved. The higher virulence of the newly discovered honeybee pathogen, *Nosema ceranae*, which causes a higher mortality in its new host *Apis mellifera*, might be based on a similar mechanism. Using Proboscis Extension Response and feeding experiments, we show that bees infected with *N. ceranae* have a higher hunger level that leads to a lower survival. Significantly, we also demonstrate that the survival of infected bees fed *ad libitum* is not different from that of uninfected bees. These results demonstrate that energetic stress is the probable cause of the shortened life span observed in infected bees. We argue that energetic stress can lead to the precocious and risky foraging observed in Nosema infected bees and discuss its relevance to colony collapse syndrome. The significance of energetic stress as a general mechanism by which infectious diseases influence host behavior and physiology is discussed.

1. Introduction

Parasites typically compete with their hosts for nutrition and exert an energetic stress on them. There are two different mechanisms by which the energetic stress is imposed, the parasite either directly draws energy from the host for its own metabolic needs or the host needs to expend energy for mounting an immunological response, which is known to be an energetically expensive process (Schmid-Hempel, 2005). The energetic stress placed on the host as a result of an infection can compromise the effectiveness of the immune response itself and allow other pathogens to invade the host, setting off a cascading effect. Such severe and continued stress might lead to complex changes in host feeding behavior as they seek to meet this nutritional shortfall (Thompson and Redak, 2008). Some pathogens such as microsporidians are particularly severe on their hosts in terms of exerting an energetic stress because they lack mitochondria and therefore have little metabolic ability themselves (Agnew and Koella, 1997).

Nosema is a microsporidian pathogen that infects the honeybee gut and is known to cause a suite of metabolic changes in the host (Bailey, 1981). Infected bees are known to have lower levels of protein, resulting in a reduced hypopharengeal gland (Malone and Gatehouse, 1998; Wang and Moeller, 1970; Wang and Moeller, 1971), as well as altered fatty acid composition in the hemolymph (Roberts, 1968). It has been less commonly suggested that *Nosema* also uses carbohydrates from the epithelial cells of the honeybee gut lining (Higes et al., 2007; Liu, 1984). The demand placed on the host with respect to carbohydrate is especially interesting because it is the most fundamental source of energy and bees, due to their high metabolic rates that come with flight (Neukirch, 1982), have a high demand for it. It is also important to note in this context that the foragers, which are likely to have the highest energetic demand, are also the ones with the highest *Nosema* load (El-Shemy and Pickard, 1989; Higes et al., 2008). The idea that *Nosema* places a substantial energetic demand on the host is supported by the observation that infected bees in cages consumed significantly more sugar–water although the lower oxygen consumption that accompanied it (Moffet and Lawson, 1975) suggests that infected bees are probably not able to utilize the extra carbohydrates.

A newly reported *Nosema* species, *Nosema ceranae*, has recently jumped hosts to the European honeybee (Higes et al., 2006) and is currently replacing *Nosema apis* throughout the world (Klee et al., 2007). The observations that *N. ceranae* causes a higher mortality than *N. apis* in caged bees despite the same pathogen load (Paxton et al., 2007) and that colonies infected with *N. ceranae* die if left untreated (Higes et al., 2008) suggest that the new species possibly has a higher virulence. While this means that *N. ceranae* could cause a particularly severe metabolic stress in its new host, there is little information on its physiological and behavioral effects in infected bees. Therefore, the major motivation for this study was to investigate if *N. ceranae* imposes an energetic demand on its host, causing infected bees to display an increased hunger and a lower survival as a direct consequence of it. We focus our study on the foragers because they are likely to incur the highest energetic stress due to an infection for the reasons discussed above.

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2. Material and methods

2.1. Forager collection

We monitored the *N. ceranae* infection status of two full-sized honeybee colonies in the field by regularly sampling foragers for the microsporidian spores. We collected returning foragers from these two colonies with a vacuum after placing a wire-mesh screen over the hive entrance and released them into a cage.

2.2. Proboscis extension response (PER) experiment

We placed each bee in a glass vial, chilled it on ice until the individual became immobile and strapped her within a 4.5 cm long plastic drinking straw with a small strip of tape on her thorax. Testing began 45 min after the last bee was strapped to allow the bees to get acclimated. The antennae of a strapped bee were touched with a droplet of sucrose and whether she responded by fully extending her proboscis – a Proboscis Extension Response (PER) – was recorded. Each bee was assayed with a concentration series of 0.1%, 0.3%, 1%, 3%, 10%, and 30% sucrose solution by weight and between every two successive concentrations, the antennae were touched with water to control for possible sensitization from repeated stimulation (Bitterman et al., 1983).

2.3. Hunger level experiment

Bees were strapped and fed 30% sucrose solution *ad libitum* every 6 h for 24 h and the amount consumed by each bee was recorded at each time point. The bees were kept in an incubator set at 25 °C and 70% RH during the entire period.

2.4. Survival experiment

After strapping, the bees were fed once with either 0 μl, 5 μl, 10 μl, 20 μl, 30 μl at the beginning of the experiment, or *ad libitum* and their survival was monitored every 6 h for 24 h. The bees were kept in an incubator similarly as in the previous experiment.

2.5. Infection status

After the conclusion of each experiment, the subjects were freeze-killed, their entire gut was removed and homogenized in water and the number of *Nosema* spores in each bee was quantified on a hemacytometer. Infected bees had a spore count of 2.5×10^5 or more (some bees had a spore count as high as 2.5×10^6 or more). The species of *Nosema* seen was confirmed using the multiplex PCR and electrophoresis method (Martín-Hernandez et al., 2007). Infected bees produced a DNA fragment length in the 218–219 bp range but no fragment lengths in the 312 bp range, indicating that *N. ceranae* was the only *Nosema* species present. None of the two fragment lengths were present in uninfected bees (negative controls).

3. Results

3.1. Proboscis extension response (PER) experiment

Infected bees were significantly more responsive to sucrose than uninfected bees in each colony tested: colony 1 (G test of independence: $G = 7.23$, $N = 228$, $P = 0.01$, Fig. 1a) and colony 2 ($G = 16.36$, $N = 390$, $P < 0.0001$, Fig. 1b), especially at the lower concentrations, indicating that infection with *N. ceranae* increased their appetite. As the difference in response between control and infected bees were consistent between the two colonies, data from them were pooled in the next two experiments.

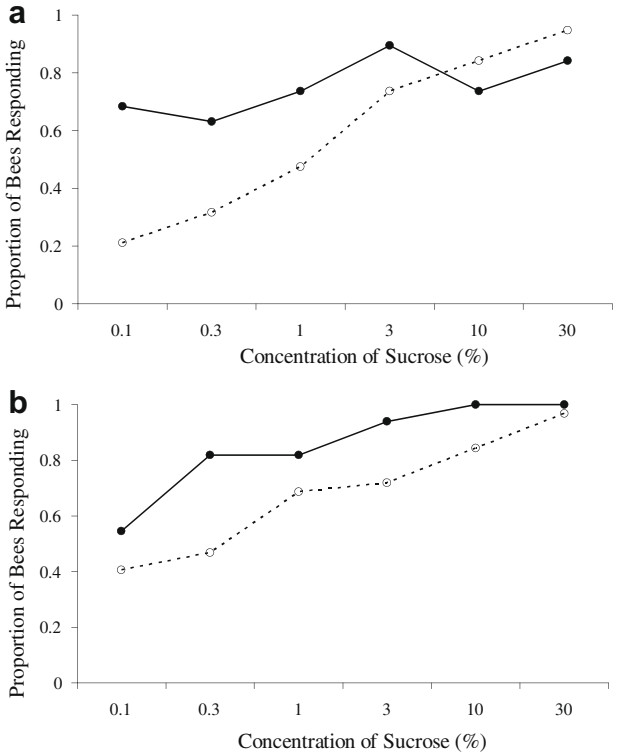

Fig. 1. Responsiveness of infected (●) and control (○) bees to sucrose solution of different concentrations in: (a) colony 1 (228 antennal probes from 19 control and 19 infected bees) and (b) colony 2 (390 antennal probes from 32 control and 33 infected bees). Proportion of responses is overall higher in colony 2 in comparison to colony 1 but the responsiveness of infected bees is higher than control bees within each colony.

3.2. Hunger level experiment

Infected bees consumed a significantly higher amount of sucrose over the 24 h period tested (repeated measures ANOVA: $F_{1,99} = 27.44$, $P < 0.0001$, Fig. 2). The amount fed by the bees significantly decreased with time ($F_{1,99} = 108.80$, $P < 0.0001$) but there was a significant interaction effect ($F_{1,99} = 5.96$, $P = 0.016$) indicating that infection not only increases overall hunger but also the rate at which bees starve.

3.3. Survival experiment

Survival of bees significantly depended on the amount of food consumed (repeated measures ANOVA: $F_{4,5} = 13.25$, $P = 0.007$, Fig. 3a), with almost no bees surviving for more than 24 h when

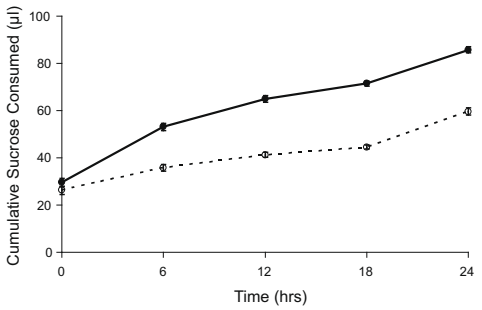

Fig. 2. Cumulative consumption of 30% sucrose solution by infected (●) and control (○) bees until satiation, measured every 6 h for 24 h. Data represent mean values for infected ($N = 57$) and control ($N = 44$) bees with standard error bars.