Nosema spp. infections cause no energetic stress in tolerant honeybees

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Abstract

Host-pathogen coevolution leads to reciprocal adaptations, allowing pathogens to increase host exploitation or hosts to minimise costs of infection. As pathogen resistance is often associated with considerable costs, tolerance may be an evolutionary alternative. Here, we examined the effect of two closely related and highly host dependent intracellular gut pathogens, *Nosema apis* and *Nosema ceranae*, on the energetic state in *Nosema* tolerant and sensitive honeybees facing the infection. We quantified the three major haemolymph carbohydrates fructose, glucose, and trehalose using high-performance liquid chromatography (HPLC) as a measure for host energetic state. Trehalose levels in the haemolymph were negatively associated with *N. apis* infection intensity and with *N. ceranae* infection regardless of the infection intensity in sensitive honeybees. Nevertheless, there was no such association in *Nosema* spp. infected tolerant honeybees. These findings suggest that energy availability in tolerant honeybees was not compromised by the infection. This result obtained at the individual level may also have implications at the colony level where workers in spite of a *Nosema* infection can still perform as well as healthy bees, maintaining colony efficiency and productivity.

Keywords

Host-parasite interaction Immune response Energetic stress Adaptation Fitness cost

Introduction

An infection or disease can lead to a severe reduction of host fitness by reducing both host survival and reproductive success (Hurd, 2001). These fitness costs to the host are not only imposed by pathogens directly but may also be a result of immune function itself (Lochmiller & Deerenberg, 2000; Schmid-Hempel, 2005). The host has to reallocate its resource availability investing more into immune responses which typically leads to life-history trade-offs (Sheldon & Verhulst, 1996). For example, an immune challenge may result in decreased survival (Moret & Schmid-Hempel, 2000), self-harm (Sadd & Siva-Jothy, 2006), or altered development and fecundity (Roth & Kurtz, 2008). Because immunocompetence is a costly process (Schmid-Hempel, 2005), natural selection would not necessarily favour fully resistant

host phenotypes, but rather tolerant hosts that can sustain parasitic infection, keeping infection intensity at bay while sustaining a high level of fitness (Råberg et al., 2007; Sorci, 2013). Thus, a well-adapted host might be able to either allocate resources more efficiently or has acquired a mechanism to compensate for any resource losses due to parasitic infection. In social insect societies such as honeybees, sterile workers contribute to the fitness of the colony without receiving direct fitness benefits (Hamilton, 1964; Moritz & Southwick, 1992). Hence, it is the fraction of infected workers in a colony which is a critical parameter in determining tolerance to an infectious disease rather than the infection level of an individual worker (Straub et al., 2015).

In antagonistic coevolution, pathogens will be selected to minimise the cost of immune clearance and counter-adapt by either escaping or at least reducing the effectiveness of the host's immune system (Schmid-Hempel, 2008). Escaping the host's immune response might be particularly important for highly specialised parasites such as Microsporidia. Because this group of intracellular fungi lacks mitochondria, they are entirely dependent on the host's energy metabolism to ensure reproduction and survival (Keeling, 2009). Microsporidia have evolved elaborate infection mechanisms in a wide range of animal hosts, which includes humans and in economically and ecologically important animals such as honeybees, silk worms, and salmon (Keeling, 2009). In particular the emerging gut pathogen *Nosema ceranae* has gained tremendous attention in recent years and is considered to be an important pathogen in honeybees worldwide (Higes et al., 2013). Similar to its native closely related sister species *Nosema apis*, spores of *N. ceranae* are horizontally transmitted by ingestion and germinate in the host's ventricular (midgut), where they penetrate and enter epithelial cells via their polar tubes. *Nosema* then exploits the host cell's metabolism for its own reproduction (Fries, 2010; Gisder et al., 2011).

Although *N. ceranae* infection does not appear to alter the navigation and orientation of the honeybees while foraging, increased foraging duration and decreased flight frequencies have been reported in infected honeybees (Alaux et al., 2014; Dussaubat et al., 2013; Naug, 2014; Wolf et al., 2014). This decline in foraging efficiency is likely to be a result of imposed nutritional and energetic stress from infection (Aliferis et al., 2012; Martín-Hernández et al., 2011; Mayack & Naug, 2009; Mayack & Naug, 2010; Moffett & Lawson, 1975). Infected honeybee workers also appear to gain energy less efficiently, which may negatively influence their lifespan and the colony performance on a long-term basis (Naug, 2014). Moreover, *N. ceranae* infection causes immunosuppression in honeybees (Antúnez et al., 2009; Chaimanee et al., 2012; Holt et al., 2013; Huang et al., 2012), which could be the consequence of energetic stress due to costly maintenance of an immune response (Schmid-Hempel, 2005).

Because *Nosema* infections are negatively associated with honeybee health, Danish honeybee queen breeders selected against *Nosema* spp. by excluding infested queens from further breeding for two decades (Hatjina et al., 2014). Not only did the *Nosema* spp. prevalence decline from 60–80 % to approximately 10 % in colonies of this tolerant strain (based on 60 workers/colony) as a result of this artificial selection (Hatjina et al., 2014), but moreover these tolerant honeybees appeared to survive *N. ceranae* infection better than *Nosema* sensitive honeybees, which could be explained by up-regulated expression levels of key immune genes (Huang et al., 2012). However, it is not known whether these selected tolerant honeybees suffer trade-offs due to resource allocation shifts. Although an increase immune response may cause additional nutritional or energetic costs to the host (Schmid-Hempel, 2005), tolerance is a mechanism allowing the host to sustain the infection and reduce the

damage induced by pathogens or overreacting immune response while paying little or even no fitness costs (Råberg et al., 2007; Sorci, 2013).

Therefore, we use haemolymph carbohydrates as an absolute measure of energy allocation and availability during the infection in this study. We compared the energetic cost associated with a *Nosema* infection between a *Nosema* tolerant and sensitive honeybee lineage. In honeybees, the disaccharide trehalose circulating in the haemolymph acts as a major energy storage source to fuel activities such as flight (Blatt & Roces, 2001; Suarez et al., 1996). In addition, the carbohydrate composition in the haemolymph reflects nutrient intake and can be used as an indicator of nutritional status in insects (Thompson, 2003). We therefore decided to measure the concentration of trehalose, glucose and fructose in the haemolymph to estimate the energetic costs incurred from a *Nosema* infection. We hypothesise that energy availability in the haemolymph is negatively associated with *Nosema* infection load in *Nosema* sensitive honeybees. In addition, we hypothesise that *Nosema* tolerant honeybees in contrast to sensitive honeybees compensate any energy depletion via to an adaptation of their energy metabolism to *Nosema* spp. infection.

Materials and methods

Honeybee hosts

Worker honeybees (*Apis mellifera*) were first reared from the selected *Nosema* tolerant (T) lineage, provided by the Department of Integrated Pest Management Research Centre Flakkebjerg, Denmark, and from a *Nosema* sensitive (S) lineage, provided by the Laboratoire des Abeilles et Environnement de l'INRA Avignon, France. Frames of capped worker brood were obtained from each lineage and kept in an incubator at 34 ± 1 °C, 60 % relative humidity and 24 h darkness. Newly emerged *Nosema* free worker honeybees (<12 h old) were collected from brood frames. They were housed in groups of 30 individuals each in autoclaved stainless steel cages (10 × 10 × 5.5 cm) and placed back into the incubator. Throughout the entire duration of the trials, honeybees were fed 50 % (*w*/*v*) sucrose solution ad libitum.

Nosema spp. isolates

For inoculations, we used *N. ceranae* spores originating from Germany and *N. apis* spores originating from Sweden. *N. ceranae* spores were freshly extracted and *N. apis* spores were obtained from frozen stock (Fries, 2010). Inocula were then purified using the triangulation centrifugation method (Fries et al., 2013). To obtain the required *Nosema* concentration, we counted *Nosema* spores using a Fuchs–Rosenthal haemocytometer placed under a phase–contrast microscope (Olympus CX41, Olympus, Hamburg, Germany) and then diluted spores to a concentration of approximately 50,000 spores μl^{-1} in 50 % (*w*/*v*) sucrose solution before inoculation. *Nosema* species identification was confirmed by PCR as described previously (Gisder & Genersch, 2013).

Experimental inoculation

Inoculation experiments were conducted for each honeybee lineage (S = sensitive and T = tolerant) at the Martin-Luther-Universität Halle-Wittenberg in Germany from May to July 2014, following standard methods for *Nosema* research in honeybees (Fries et al., 2013). Three day old bees were either individually fed with approximately 10^5 *N. ceranae* (SNc and

TNc) or *N. apis* (SNa and TNa) spores in 2 µl of 50 % (*w*/*v*) sucrose solution to induce successful infections (ID_{100}) (Forsgren & Fries, 2010; Fries, 2010). Negative controls (SC and TC) were individually fed with 2 µl of pure sucrose solution. Only bees consuming the entire inoculum were included in the experiments. We chose to terminate the experiment at a relatively early stage of the infection on 6 days post infection (p.i.), when infection had been established and the first generation of spores had been produced (Fries et al., 1992; Gisder et al., 2011), to obtain individuals facing infections ranging from low to high intensities and to make our results comparable with previous experiments (Huang et al., 2012; Kurze et al., 2015). Haemolymph was obtained from approximately five randomly chosen individuals per treatment group per replicate, and the digestive tract was immediately dissected out of the bee. Four replicates were conducted per treatment group, except for *N. apis* infected tolerant honeybees with only three replicates.

Haemolymph extraction

Haemolymph collection was performed according to standard methods (Hartfelder et al., 2013). Briefly, bees were immobilised by crossing two pins over the waist of the bee mounted on a wax-filled Petri dish. Then, 2 µl of haemolymph was collected from a small incision on the side of the bee's abdomen using a glass microcapillary (Brand, Wertheim, Germany). Obtained haemolymph was subsequently snap frozen in liquid nitrogen and then stored at −80 ° C until HPLC analysis.

Nosema spore count

Both *N. ceranae* and *N. apis* are known to only infect the ventriculus (i.e. midgut without rectum) tissue of the honeybee (Huang & Solter, 2013). Thus, midguts of at least five randomly chosen bees per treatment group and replicate were individually homogenised in 1 ml distilled water and the numbers of mature spores were counted with a Fuchs–Rosenthal haemocytometer placed under a phase–contrast microscope (×400).

Determination of sugar levels

For the identification and quantification of the three sugars found in honeybee haemolymph (glucose, fructose, and trehalose), we used high-performance liquid chromatography (HPLC). In addition, sucrose was measured to serve as an indicator of crop contamination which might have occurred during the haemolymph extraction. Prior to HPLC analysis, 2 µl of each haemolymph sample were mixed with 22 µl acetonitrile (CH_3CN) + distilled water (8 + 2 *v*/*v*), kept on ice and centrifuged at 10,000 g for 6 min maintained at 4 ° C (Eppendorf centrifuge 5804 R, Eppendorf, Germany) (Bozic & Woodring, 1997).

Then 20 µl of the final supernatant per sample was transferred to microvials with glass inserts and subsequently 10 µl was separated at 30 °C constant temperature using a 5-µm Nucleosil-100 NH_2 column (250 × 4 mm^2, Knauer, Berlin, Germany) in the HPLC Agilent 1100 series system with autosampler (Agilent Techologies, Waldbronn, Germany). The flow rate was set at 1 ml min^{-1}.

For separation of carbohydrates, the following elution programme based on (A) acetonitrile + water (87 + 13 *v*/*v*) and (B) acetonitrile + water (50 + 50 *v*/*v*) was used: 0 min 100 % (A), 10 min 87 % (A), 18 min 80 % (A), 19 min 50 % (A), 23 min 50 % (A), 24 min 100 % (A), and 30 min 100 % (A). The system was calibrated after every 20 samples using at

least five levels of standard solutions containing a mixture of trehalose, glucose, fructose, and sucrose at varying concentrations to cover the entire biological realistic range of possible sample concentrations.

Detection of carbohydrates was accomplished with a SEDEX 55 evaporative light-scattering detector (S.E.D.E.R.E., Altertville Cedex, France). The detector settings were kept constant throughout the HPLC analyses, the evaporation temperature was set at 75 °C and the nitrogen pressure at 1.9 bar. Data were acquired and analysed with the ChemStation A08.03 software (Agilent Technologies). All results were converted into mg ml^{-1}. If a sample contained more than 0.5 mg ml^{-1} sucrose, this was considered as a sign of contamination and these samples were not included in the analysis (Woodring et al., 1993).

Statistical analysis

Statistical analyses and data plotting were performed in R version 3.2.1 (R Development Core Team, 2015). The numbers of *N. apis* and *N. ceranae* spores were $\log_{10}$ transformed for all statistical tests. Differences in spore loads between treatment groups were tested using a Kruskal–Wallis one-way ANOVA, followed by pairwise comparisons using the Wilcoxon rank sum test. Multiple testing was accounting with Bonferroni adjustments. Depending on the data distribution, we tested the effect of treatment and honeybee lineages and their interactions on haemolymph sugar level, using a Kruskal–Wallis one-way ANOVA for glucose and fructose and a two-way ANOVA for trehalose levels. Post hoc analyses between treatment groups were performed using the glht function with the Bonferroni adjustment (multcomp package, v.1.4-1). Linear regression analyses were computed between the $\log_{10}$ number of *Nosema* spp. spores and the haemolymph trehalose concentrations for *Nosema* sensitive and tolerant honeybees. Differences between slopes were calculated by using MANCOVA. To avoid any statistical bias, we inspected residuals, leverage and Cook's D and tested for potential outliers with Bonferroni correction.

Results

Infection load

The mean $\log_{10}$ number of spores ± standard deviation (s.d.) in sensitive and tolerant honeybees were 5.46 ± 0.82 (SNa, $n = 20$) and 4.73 ± 0.76 (TNa, $n = 15$), respectively, for *N. apis* infections and 5.61 ± 0.42 (SNc, $n = 22$) and 5.23 ± 0.83 (TNc, $n = 22$), respectively, for *N. ceranae* infections. All screened controls (SC, $n = 20$; TC, $n = 23$) were free of *Nosema* spp. infection. Although there was a significant effect between treatment groups (Kruskal–Wallis: $\chi^2 = 11.675$, df = 3, $p = 0.01$), where the $\log_{10}$ spore load in SNc was significantly higher compared to TNa (Wilcoxon $p < 0.01$), treatment groups did not otherwise not significantly differ in their $\log_{10}$ spore loads from each other.

Haemolymph sugar concentrations

We found no significant differences in haemolymph sugar concentrations between treatment groups for glucose (Kruskal–Wallis: $\chi^2 = 3.376$, df = 5, $p = 0.642$, Fig. 1a) and fructose (Kruskal–Wallis: $\chi^2 = 9.827$, df = 5, $p = 0.080$, Fig. 1b). However, there was a significant interaction between honeybee lineage and treatment affecting trehalose concentrations (ANOVA: $F_{2, 142} = 7.740$, $p < 0.001$; Fig. 1c) whereby *N. ceranae* infection was associated with reduced haemolymph trehalose availability in *Nosema* sensitive honeybees (SNc:

mean ± 95 % CI = 11.0 ± 3.4 mg/ml, $n = 23$) compared to tolerant honeybees (TNc: 24.9 ± 3.4 mg/ml, $n = 27$; $p < 0.001$). Furthermore, trehalose concentrations in SNc were significantly lower compared to SNa (19.1 ± 4.2 mg/ml, $n = 27$; $p < 0.05$) and SC (18.4 ± 4.3 mg/ml, $n = 25$; $p < 0.05$). Nevertheless, trehalose concentrations in TNc and TNa (21.4 ± 4.4 mg/ml, $n = 17$) were not significantly different compared to their controls (TC: 19.7 ± 2.7 mg/ml, $n = 17$; TNc-TC: $p = 0.18$; TNa-TC: $p > 0.05$). There was also no significant difference of trehalose concentrations between uninfected controls of both honeybee lineages (SC, TC; $p > 0.05$).

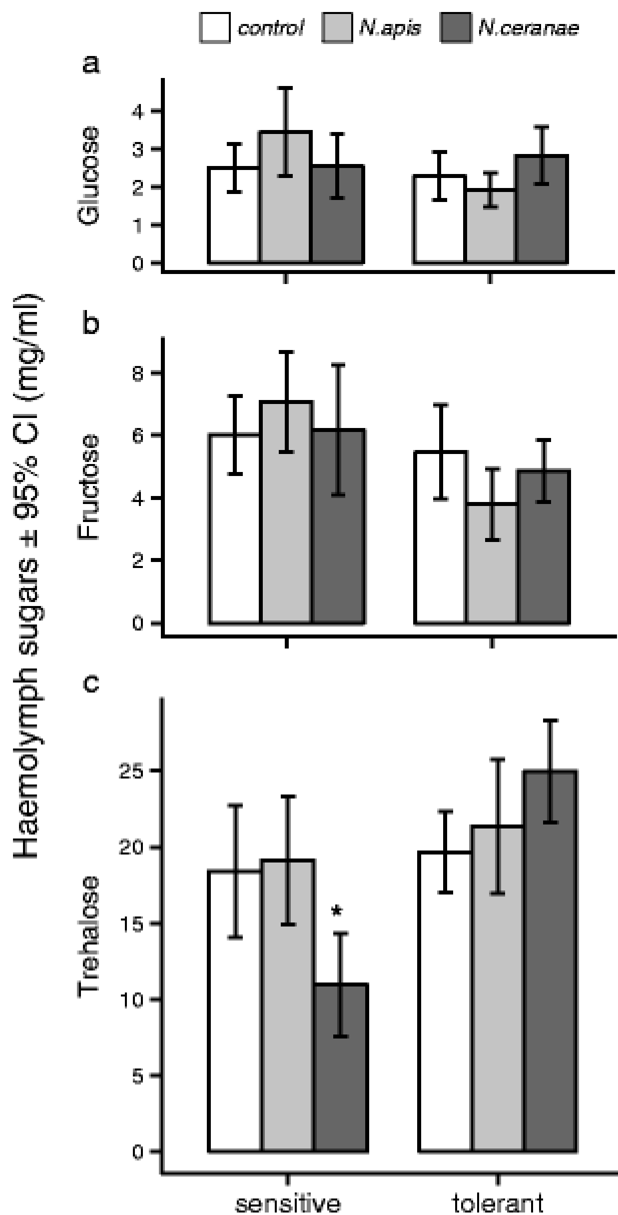

Fig 1 : Effect of *Nosema* spp. infection on haemolymph sugar levels in *Nosema* sensitive and tolerant honeybees 6 days after infection. Mean sugar concentrations ± 95 % confidence intervals (CI) for glucose (**a**), fructose (**b**), trehalose (**c**) in uninfected controls (*white*), *N. apis* (*light grey*) and *N. ceranae* (*dark grey*) infected sensitive (*left*; $n = 25, 27, 23$) and tolerant strain honeybees (*right*; $n = 29, 17, 27$). Significant differences $p < 0.05$ are indicated by *asterisks*

Regression between spore load and haemolymph sugars

Linear regression analyses revealed a highly significant negative association between infection load and trehalose titres for *N. apis* infected sensitive honeybees (SNa: adjusted $R^2 = 0.497$, $F_{1, 18} = 17.75$, s.e. of estimate = 8.729, $p < 0.001$; Fig. 2a), but not for *N. ceranae* infected sensitive honeybees (SNc: adjusted $R^2 = -0.049$, $F_{1, 20} = 0.014$, s.e. of estimate = 8.159, $p = 0.908$; Fig. 2a). Moreover, we found neither in *N. apis* nor *N. ceranae* infected tolerant honeybees such association (TNa: adjusted $R^2 = -0.035$, $F_{1, 13} = 0.526$, s.e. of estimate = 7.793, $p = 0.481$; TNc: adjusted $R^2 = -0.047$, $F_{1, 20} = 0.058$, s.e. of estimate = 9.023, $p = 0.812$, Fig. 2b). The difference observed on trehalose availability was supported by a significant interaction between *Nosema* spp. and $\log_{10}$ spore load (MANCOVA: $F_{3, 38} = 10.848$, s.e. of estimate = 5.043, $p < 0.05$).

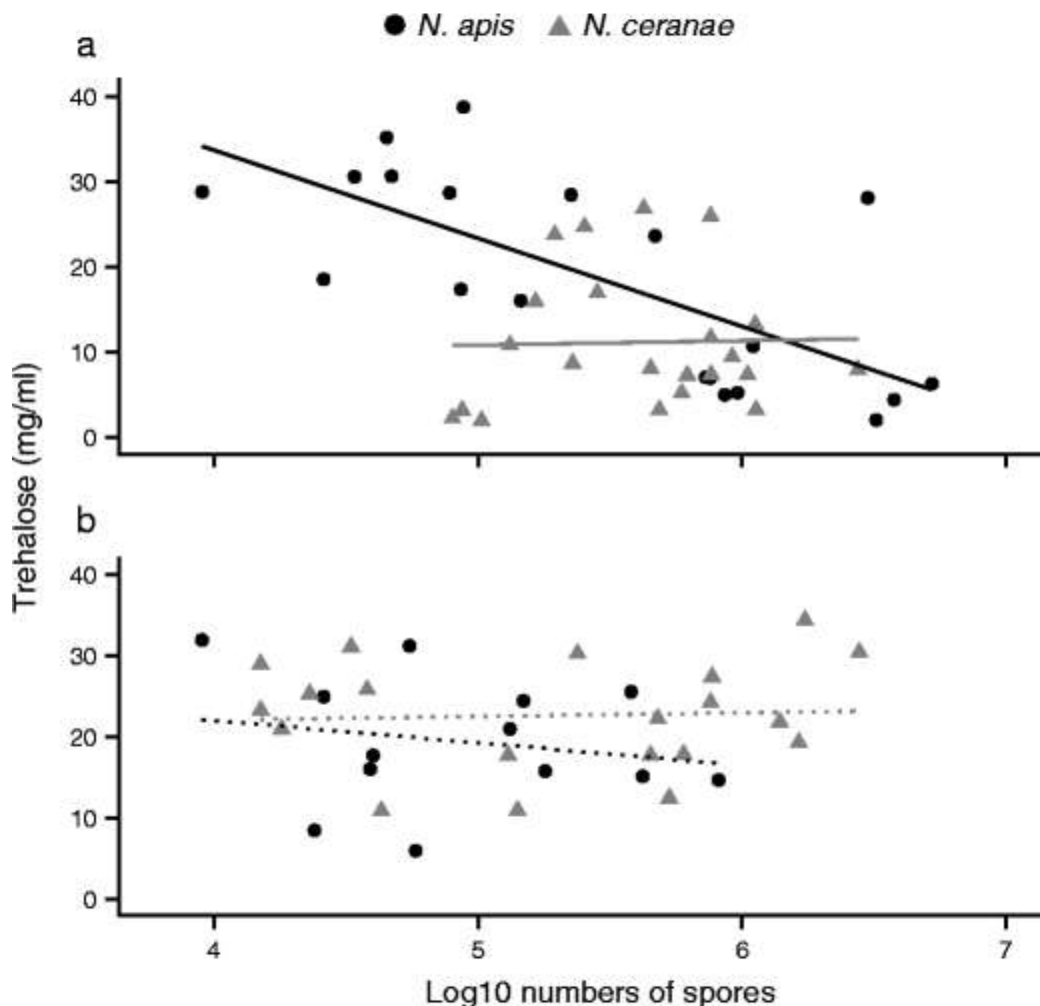

Fig 2 : Relationship between $\log_{10}$ transformed *N. apis* (*black circles*) and *N. ceranae* (*grey triangles*) spore loads and haemolymph trehalose concentrations in *Nosema* sensitive (**a**) and tolerant (**b**) honeybees after 6 days of infection. Each data point represents the measurements for a single honeybee. The lines in the graphs are regression lines: (**a**, *black*) adjusted $R^2 = 0.497$, $F_{1, 18} = 17.75$, s.e. of estimate = 8.729, $p < 0.001$; (**a**, *grey*) adjusted $R^2 = -0.049$, $F_{1, 20} = 0.014$, s.e. of estimate = 8.159, $p = 0.908$; (**b**, *black*) adjusted $R^2 = -0.035$, $F_{1, 13} = 0.526$, s.e. of estimate = 7.793, $p = 0.481$ and (**b**, *grey*) adjusted $R^2 = -0.047$, $F_{1, 20} = 0.058$, s.e. of estimate = 9.023, $p = 0.812$

Discussion

Our study revealed that declining levels of trehalose in the haemolymph correlate with increasing *N. apis* spore load in sensitive honeybees. Although *N. ceranae* infections were also associated with reduced trehalose levels in sensitive honeybees, we did not find such correlation between trehalose levels and spore loads. This could possibly be explained by the limited infection range, as we have not detected any individuals from the sensitive lineage with low *N. ceranae* infection intensities. From our data, worker bees with medium infection loads appeared as energetically compromised as those with high infection levels. This and the fact that we did not detect workers with low spore loads in our samples may reflect a higher virulence of *N. ceranae*. On the one hand, it seems that *N. ceranae* elicits a high metabolic response at lower spore load than *N. apis* does, indicating higher energetic costs for workers to fight an infection with *N. ceranae*. On the other hand, spore load may have increased more rapidly in *N. ceranae* than in *N. apis*, which could alternatively be explained by differences between inocula. Despite this, our results would support the notion of higher virulence due to a relatively recent host-parasite relationship compared to *N. apis*, which has a long host-parasite coevolutionary history (Higes et al., 2007; Martín-Hernández et al., 2011; Williams et al., 2014). Nevertheless, our findings confirm that *Nosema* infections are depleting the amount of available trehalose present in the haemolymph (Mayack & Naug, 2010), which may be responsible for the reduced survival of infected sensitive honeybees (Dussaubat et al., 2012; Higes et al., 2007).

In contrast, we found no such association over a comparable range of either *N. apis* or *N. ceranae* spore loads with trehalose levels in the haemolymph of infected honeybees of tolerant lineage. Apparently, these bees were on average able to maintain the normal energy availability as the uninfected controls. Although we cannot rule out that *Nosema* will not have any effect on tolerant host at much higher infection intensities, but our data may provide an explanation why their survival was not affected in inoculation experiments (Huang et al., 2012). It is important to note that a few individuals displayed relatively low trehalose levels in the haemolymph even at lower infection intensities. This observation may be explained by the intrinsic high variability of sugar levels and metabolic rates found in honeybees (Fell, 1990; Harrison & Fewell, 2002) or alternatively this may indicate cost of immune defence in these individuals. Nevertheless, selective breeding for *Nosema* absence has not only resulted in genetically distinct honeybees (Huang et al., 2014a; Huang et al., 2014b) that appear to have acquired a tolerance mechanism to keep *N. ceranae* at bay (Kurze et al., 2015) but they also appear to maintain sufficient energy stores as well. Because suppression of the acute immune response in sensitive honeybees might be the consequence of the energetic stress imposed by a *Nosema* infection (Antúnez et al., 2009; Holt et al., 2013), the maintenance of high trehalose levels in *Nosema* tolerant honeybees might explain the preservation of immune responses found in these bees when challenged with a *N. ceranae* infection (Huang et al., 2012; Kurze et al., 2015).

Haemolymph trehalose serves as a major energy store in honeybees and other insects, providing rapid energy availability essential for flight (Suarez et al., 2005; Suarez et al., 1996; Thompson, 2003). As trehalose synthesis is energetically costly (Thompson, 2003), it is thought to be the limiting step in the maintenance of high trehalose levels in honeybees because its synthesis cannot keep up with its degradation when energy demand is high (Woodring et al., 1994). Honeybees maintain low glucose and fructose concentrations in the haemolymph at the expenses of trehalose, especially if their metabolic rates are high, like when flying during foraging trips (Blatt & Roces, 2001). This would plausibly explain why

we did not detect any significant alterations in glucose and fructose levels in infected honeybees relative to uninfected bees as shown by Aliferis et al., (2012). As the haemolymph concentration of glucose, fructose, and trehalose levels in uninfected control honeybees in our study were within the expected range presented in previous physiological studies (Blatt & Roces, 2001; Woodring et al., 1993), we are confident of the reliability of our HPLC measurements and the mechanistic representation of the influence of *Nosema* infection on the energy demand at individual level of the bee.

As the chemosensory sensitivity of taste receptors in insects is linked with the concentration of haemolymph carbohydrates (Thompson, 2003), it is suspected that decreasing trehalose levels lead to an elevated appetite responsiveness in *Nosema*-infected sensitive honeybees (Mayack & Naug, 2009). Although this would indicate increased hunger in those honeybees, *Nosema*-infected workers did not consume more food despite increased mortality (Williams et al., 2014). However, decreased trophallaxis (food sharing) in *Nosema*-infected honeybees (Naug & Gibbs, 2009), suggests that energetically stressed honeybees are not likely to share food with their nest mates. Instead, *Nosema*-infected honeybees actually leave the hive energetically stressed (Mayack & Naug, 2010) and may not return (Wolf et al., 2014). This behavioural alteration of infected honeybees might also reduce the parasite transmission and ultimately limit the damage at the colony level. However, changes in the foraging behaviour of infected individuals is also likely to negatively affect the life-history traits of the colony (Alaux et al., 2014; Naug, 2014; Wolf et al., 2014) and lower its overall fitness as a whole in the long run.

Nosema tolerant honeybees might be able to compensate the energetic losses due to the infection by modifying their behaviour in terms of food consumption or activity. Therefore, the actual energetic cost of mounting the immune response against the *Nosema* infection (Huang et al., 2012; Lochmiller & Deerenberg, 2000) might not be relevant in the tolerant honeybees. This is also supported by data showing that better alimentation affected host survival positively regardless the severity of the *Nosema* infection (Jack et al., 2016).

Although resource allocation makes it also difficult to disentangle whether costs are caused by the parasite directly or by the immune defence (Rauw, 2012), our individual-based data suggests that the energy imbalance in the *Nosema* sensitive honeybee lineage is caused by *Nosema* spp. directly rather than by the host's immune response. Firstly, because trehalose levels were not significantly negatively affected in the infected tolerant honeybees, even though they show an increased immune response (Huang et al., 2012). This may also imply that the cost of immune response as such might be reduced as consequence of tolerance which is working alongside with an increase in efficiency of a refuelling mechanism. Secondly, microsporidia are known to be highly dependent on the ATP as energy source from the host as they can only obtain ATP themselves via glycolysis (Keeling, 2009; Williams, 2009) and we found a negative association between available trehalose in the haemolymph and the infection load of *N. apis* in sensitive honeybees.

In conclusion, our results clearly show that *Nosema* infection leads to an energy imbalance in sensitive honeybee workers, but not in the *Nosema* tolerant honeybees. These tolerant honeybees appear to have acquired a mechanism to maintain haemolymph carbohydrate homeostasis when food is available ad libitum. Possibly, this trait might play a key role in the maintenance of their immune response towards *Nosema* spp. and could help in explaining their advantage to fight and withstand the disease in contrast to *Nosema* sensitive honeybees. The energetic state might not only be important for the individual honeybee during a

pathogen-challenge, but is also likely to affect the overall fitness of the colony as a whole. The energetic stress caused by the *Nosema* spp. on an individual level is therefore likely to scale-up and have major implications on the general performance of the entire colony.

Acknowledgments

We thank three anonymous reviewers for their constructive comments, which helped to improve our manuscript. The study was supported by the Deutsche Forschungsgemeinschaft DFG priority programme SPP 1399 "Host-parasite co-evolution" (grant number MO373/26-2).

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