Horizontal transmission success of *Nosema bombi* to its adult bumble bee hosts: effects of dosage, spore source and host age

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SUMMARY

Parasite transmission dynamics are fundamental to explaining the evolutionary epidemiology of disease because transmission and virulence are tightly linked. Horizontal transmission of microsporidian parasites, e.g. *Nosema bombi*, may be influenced by numerous factors, including inoculation dose, host susceptibility and host population heterogeneity. Despite previous studies of *N. bombi* and its bumble bee hosts, neither the epidemiology nor impact of the parasite are as yet understood. Here we investigate the influence *N. bombi* spore dosage (1000 to 500 000 spores), spore source (*Bombus terrestris* and *B. lucorum* isolates) and host age (2- and 10-day-old bees) have on disease establishment and the presence of patent infections in adult bumble bees. Two-day-old bees were twice as susceptible as their 10-day-old sisters, and a 5-fold increase in dosage from 100 000 to 500 000 spores resulted in a 20-fold increase in the prevalence of patent infections. While intraspecific inoculations were 3 times more likely to result in non-patent infections there was no such effect on the development of patent infections. These results suggest that host-age and dose are likely to play a role in *N. bombi*'s evolutionary epidemiology. The relatively low levels of horizontal transmission success are suggestive of low virulence in this system.

Key words: horizontal transmission, dosage, age-effect, cross-infection, epidemiology, *Nosema bombi*, *Bombus* spp.

INTRODUCTION

The epidemiology of host-parasite systems is often far from being understood. Partly, this can be attributed to a lack of understanding of transmission dynamics and their consequences. Specific mechanisms of parasite transmission may play a crucial role in explaining the evolutionary epidemiology of a disease. For example, in host-parasite systems with mixed transmission routes, the relative importance of horizontal versus vertical transmission can be a key determinant in the evolution and maintenance of virulence (Herre, 1993; Ebert and Herre, 1996; Lipsitch *et al*. 1996; Dunn and Smith, 2001). An understanding of these epidemiological dynamics is thus crucial both to test theory and to be able to manipulate and control disease.

It is generally expected that increased opportunities for horizontal transmission can lead to the evolution of higher pathogenicity, while increased levels of vertical transmission are predicted to favour reduced virulence (e.g. Bull *et al*. 1991; Bull, 1994; Ebert, 1999; Dunn *et al*. 2000). The efficiency and relative importance of horizontal transmission can be influenced by a variety of factors and their interactions. Among others, these factors include ecological traits such as inoculation dose (Bailey and Ball, 1991; Malone *et al*. 2001) and history of exposure (Nowak and May, 1994; May and Nowak, 1995; Frank, 1996; Allander and Schmid-Hempel, 2000), as well as host susceptibility (Gandon and Michalakis, 2000; Doums *et al*. 2002) and heterogeneity of the host population (Ebert, 1994, 1998; Morand *et al*. 1996; Regoes *et al*. 2000; Ganusov *et al*. 2002; Hatcher *et al*. 2005).

Nosema bombi is a microsporidian parasite of bumble bees (Fantham and Porter, 1914; McIvor and Malone, 1995). Infections by *N. bombi* have been reported in a number of different bumble bee species (Fantham and Porter, 1914; MacFarlane *et al*. 1995; Tay *et al*. 2005; Larsson, 2007) and occur at prevalences of up to 50% and 55% among males and workers respectively (Shykoff and Schmid-Hempel, 1991). Transmission stages of the parasite are released in the faeces of adult bees, and then presumably consumed by new hosts. Despite numerous studies, however, neither the epidemiology of the parasite nor its impact on its host are as yet understood. Horizontal transmission of *N. bombi* has been claimed to be restricted to the larval stage of the host by some authors (Eijnde and Vette, 1993), or to occur

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in both larvae and adults (McIvor and Malone, 1995; Schmid-Hempel and Loosli, 1998). *Nosema apis*, the equivalent microsporidian in honey bees, is only able to infect adult workers (Bailey, 1955, 1981; Hassanein, 1951). Field studies suggest that horizontal transmission occurs between bumble bee colonies, presumably via either flowers (when infected worker bees leave spores behind after collecting nectar or pollen) or worker drifting (Imhoof and Schmid-Hempel, 1999), and recent genetic data suggest that such colony-to-colony horizontal transmission has resulted in a lack of parasite population structure across its multiple bumble bee host species (Tay *et al*. 2005), and presumably the evolution of a generalist parasite. In addition to horizontal transmission, *N. bombi* must be transmitted vertically from one generation to the next by hibernating queens, as bumble bees have an annual life-cycle (Schmid-Hempel, 2001).

While the transmission routes of *N. bombi* remain unclear, the impact it has on its host (its virulence) is even more opaque. Previous studies have suggested that *N. bombi* is associated with increased sexual productivity (Imhoof and Schmid-Hempel, 1999) or no impact at all (Betts, 1920; Fisher and Pomeroy, 1989; Shykoff and Schmid-Hempel, 1991; McIvor and Malone, 1995; Whittington and Winston, 2003). In stark contrast, other studies have found that the parasite reduces host life-span (Fantham and Porter, 1914; Schmid-Hempel and Loosli, 1998), reduces sexual productivity (Eijnde and Vette, 1993), and paralyses queen abdomens, preventing copulation (De Jonghe, 1986; MacFarlane *et al*. 1995). It has even been suggested that *N. bombi* may have caused a major population crash in commercially reared bumble bees, as well as premature colony death in commercial greenhouses in North America (Whittington and Winston, 2003).

Previous studies of horizontal transmission in this system have been conducted using single doses and sources of *N. bombi* spores, and single age cohorts or non-age-controlled groups of host animals. However, studies in other microsporidia suggest that dosage and host age are important in determining the success of transmission. Increasing dose leads to increasing prevalence or effect of infection in microsporidia that infect larval stages of their host (Inglis *et al*. 2003; Down *et al*. 2004), and in *N. apis* which infects adult honey bees (Fries, 1988; Malone *et al*. 2001), but not in a parasite of non-larval *Daphnia* (Vizoso and Ebert, 2005). Larvae become less susceptible to microsporidian parasites as they age (Novotny, 1991; Inglis *et al*. 2003; Down *et al*. 2004), and *N. bombi* has been suggested to be more infective to larval than adult bumble bees (Eijnde and Vette, 1993; but see Schmid-Hempel and Loosli, 1998).

Cross-species transmission is another potentially important aspect of horizontal transmission in this system. While recent genetic studies suggest a lack of specific interactions between *N. bombi* and its various bumble bee hosts (Tay *et al*. 2005), previous studies have suggested species-specificity exists in relation to cross-infection (De Jonghe, 1986; Schmid-Hempel and Loosli, 1998). Both studies noted differences in susceptibility among host species, however, De Jonghe (1986) noted higher susceptibility in the foreign host species while the reverse tendency was reported by Schmid-Hempel and Loosli (1998).

Finally, previous work has not distinguished between patent and non-patent infections, with most assessments of susceptibility and infection success being based on the presence of *N. bombi* spores in sectioned host tissue or host abdominal homogenate (e.g. McIvor and Malone, 1995). To understand the impact of factors such as spore dose, source and host age, on horizontal transmission, a clear distinction must be made between the establishment of an infection in a host bee, and the development of that infection into a patent, transmissible stage.

Here we investigate the influence of *N. bombi* spore dosage, spore source, and host age on the efficacy of horizontal transmission among adult bumble bees and the presence of patent infections. All of these factors are likely to influence horizontal transmission success. Because parasite transmission and virulence are tightly linked, an understanding of transmission should shed light on the evolutionary epidemiology of this system.

MATERIALS AND METHODS

Experimental animals and maintenance

Worker bees from 5 commercially raised colonies of *Bombus terrestris* (purchased from Koppert Ltd, through Hortico Ireland Ltd) served as experimental hosts in the first experiment. Commercially reared colonies come from stock that is out-bred (Ruiz-González and Brown, 2006) and to which new field-caught lines are regularly added (Velthuis and van Doorn, 2006). Thus, there is no *a priori* reason to believe that their resistance to parasitism should be different from wild bees. Furthermore, previous work with other parasites (e.g. Logan *et al*. 2005) has found no differences in the success of these parasites in commercial colonies. Because infection success in the first experiment was extremely low, 2 additional colonies were purchased from the same supplier and used as the worker source for a second follow-up experiment with increased dosages.

Prior to experiments all colonies were checked to ensure that they carried no natural parasite infections (for each colony 5 randomly selected bees were dissected and thoroughly examined for parasites – no bee was found to be infected by either *Nosema bombi* or any of the other known parasites of *B. terrestris*; Schmid-Hempel, 2001) and were transferred to

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observation hives for ease of handling (adapted from Pomeroy and Plowright, 1980). This also ensured that experimental bees never fed on treated sugar water (commercially-reared colonies are supplied with sugar water that may contain anti-fungal additives). Colonies were kept under standard rearing conditions (26 °C; 60% R.H.). Colonies were left for approximately 3 weeks before callow workers were sourced from the colonies in order to ensure that only workers that had not come in contact with treated sugar water were used in the experiments. Callow bees were removed daily, and for each colony sequentially allocated to experimental groups. Experimental animals were kept individually in small plastic boxes (12 × 10 × 7 cm) supplied with pollen and sugar water *ad libitum* and at room temperature (min = 17·8 °C max = 21·2 °C; average 18·8 °C; measurements taken with a Barigo max/min digital thermometer) under natural light conditions.

Inoculum preparation and administration

Two types of inoculum were used. Each type consisted of *N. bombi* spores that were isolated from either *B. terrestris* or *B. lucorum* queens that had been caught in the wild around Dublin, Ireland, and which had heavily infected fat-bodies and Malpighian tubules. To obtain spore isolates the whole abdomen of an infected bee was homogenized in 0·5 ml of 0·01 M NH_4Cl (ammonium chloride inhibits the premature germination of spores; Undeen and Avery, 1988) using a glass mortar and pestle. The resulting spore solution was then washed through gauze with 0·01 M NH_4Cl to separate out the remaining exoskeleton and hairs and distributed in 5 ml volumes to a series of 15 ml tubes which were subsequently centrifuged at 1000 ***g*** for 10 min. The lowermost white part of the resulting pellet was then resuspended in 0·01 M NH_4Cl and centrifuged again. This process was repeated until the remaining pellet appeared pure white. The purified pellet was then resuspended in 0·01 M NH_4Cl, counted in a haemocytometer (Neubauer chamber), and diluted to the desired spore concentration. The *terrestris* inoculum consisted of a mixture of 3 different spore isolates that originated from 3 *B. terrestris* queens caught in the spring of 2005. The *lucorum* inoculum contained a mixture of spore isolates from 3 *B. lucorum* spring queens caught in the spring of 2004. Inocula were prepared from a mixture of isolates in order to standardize inoculations and to test for species-level, rather than strain-level effects of spore origin. Inocula were stored in the freezer at −80 °C until use in the experiments. All bees were starved for 4 h before receiving their respective treatments. The inocula were administered in the form of 10 μl droplets, containing the respective amount of spores suspended in diluted sugar water, dispensed onto the floor of small plastic vials in which starved bees were confined; in every case spore suspensions were quickly consumed by the starved bee.

Experiment 1

This experiment tested the effects of dosage, spore source, and single versus multiple inoculations. In the first part, workers of 2 different age groups, 2 and 10 days old, were inoculated with 1 of 3 different dosages: low = 1000, medium = 10 000 and high = 100 000 spores per 10 μl of the *terrestris* inoculum. Dosage levels used in this study were based on results from previous investigations which indicate that dosages of ⩾60 000 spores are capable of causing infection (Schmid-Hempel and Loosli, 1998; McIvor and Malone, 1995; Rutrecht and Brown, unpublished data). To date, no study has investigated a possible threshold dosage for disease establishment. Consequently, in this experiment dosages lower than those previously examined were used to test for such a threshold. Ten individuals from each colony ($N = 5$ colonies) were allocated to each age/dosage category (total $N = 300$ bees). Secondly, to investigate possible cross-species effects, a further set of 10 workers per colony was inoculated with a single high dosage (100 000 spores per 10 μl) of the *lucorum* inoculum at 2 days of age ($N = 50$ bees). Since environmental transmission of *N. bombi* is thought to occur via spores deposited on flowers (Durrer and Schmid-Hempel, 1994; Imhoof and Schmid-Hempel, 1999), a foraging individual is likely to encounter spores repeatedly. Thus, a trickle dose may be more comparable to natural exposure. Consequently, in the third part of this experiment, an additional set of 10 workers per colony was inoculated at 2 days of age with a trickle dose of *terrestris* spores, consisting of a single low dosage (1000 spores per 10 μl) administered on 5 consecutive days (5000 spores in total).

Experiment 2

On the basis of results from Exp. 1, all workers used in the second experiment were 2 days old at infection. Ten workers each per colony ($N = 2$ colonies) were inoculated with a single dose of 500 000 *lucorum* spores per 10 μl of inoculum. Two additional sets of 10 workers per colony were inoculated with a trickle dose of *lucorum* or *terrestris* spores consisting of a single dosage (100 000 spores per 10 μl) administered on 5 consecutive days (500 000 spores in total).

Assessment of infection success

McIvor and Malone (1995) suggested that mature spores are produced within 5 days of infection (although they did not check for the presence of these spores in faeces, which would be indicative of patent infections), whereas Schmid-Hempel and Loosli

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Table 1. Numbers of infected and non-infected individuals as determined by molecular analyses of homogenized abdomens 21 days after inoculation with *Nosema bombi*

Treatment group (10^5 spores) spore source; host age	Colony 1		Colony 2		Total	
	healthy	infected	healthy	infected	healthy	infected
terrestris ; age 2*	2	8	2	6	4	14
terrestris ; age 10	5	5	9	1	14	6
lucorum ; age 2	8	2	7	3	15	5

* Two individuals in colony 2 were lost from the experiment.

(1998) observed that it took 21 days for infections to become patent (although in their later assessments of infection success and intensity, animals were not explicitly checked for the presence of patent infections). Consequently, in this study infection success was evaluated as the presence of patent infections at 2 time-points post-inoculation (p.i.). At 10 days p.i. faecal samples were taken from each inoculated animal and thoroughly examined for *N. bombi* spores under a phase-contrast microscope at 400× magnification. At 21 days p.i. (in the case of trickle infections post-inoculation refers to the last administered inoculate i.e. 26 days after the first inoculation) animals were dissected and the entire contents of the hindgut (i.e. faeces) and subsamples of the fat-body and Malpighian tubules were thoroughly examined, again under a phase-contrast microscope at 400× magnification.

Due to extremely low levels of patent infections in Exp. 1, a subsample of individuals was assessed using a sensitive molecular *N. bombi* detection method, which detects spores as well as non-spore developmental stages of the parasite in bumble bee tissue (Klee *et al*. 2006). Molecular analyses were conducted following the protocol developed by Klee *et al*. (2006) that indicates the presence of *N. bombi* by amplifying a 118 or 122 bp section of the ITS, the 3′ end of the SSU rRNA and the 5′ end of the LSU rRNA.

For bees that were visibly infected at dissections (in all of these cases spores were present in the faecal contents, indicating patent infections) infection intensities were assessed by spore counts of homogenized abdomens. Abdomens were homogenized in 0·5 ml of detergent (20 mM Tris-HCl, pH 7·5, 150 mM NaCl 1 mM EDTA, 1 mM EGTA, 1% NP-40) with a glass mortar and pestle. Each abdomen was ground with the pestle 20 times in order to standardize the procedure. Detergent instead of water was used to facilitate the release of spores from the infected tissues, and, thus, to obtain a more homogenous suspension. For the quantification of infection intensities the resulting spore suspension was diluted by half and counted in a haemocytometer (Neubauer chamber) under the microscope at 400× magnification.

Statistical analyses

Differences in the proportion of individuals infected by *N. bombi* among the various treatment groups (dosage, age, single/trickle dose) were assessed using Fisher's exact tests. Data for spore intensities were analysed using a 2-way ANOVA and one-sample *t*-tests. All analyses were conducted on SPSS 12 for the PC and SPSS 13 for the Mac. Results were considered significant at $P<0{\cdot}05$, and two-tailed tests of significance were used throughout.

RESULTS

Experiment 1

Out of a total of 392 individual *B. terrestris* workers (8 bees from various treatment groups escaped and were lost from the experiment), only a single bee, which was treated at age 2 with a single high dosage (100 000 spores) of *lucorum* spores, was found to be visually infected at dissection. No spores were found in the 392 faecal samples taken at day 10 p.i. Thus, the proportion of patent infections across the entire experiment was extremely low (0·25%). The single infected worker carried ∼3·7 million spores.

In order to determine whether non-patent infections had been established, a subset of 3 treatment groups (high dosage *terrestris* spores for age groups 2 and 10, and high dosage *lucorum* spores at age 2) from the colony from which the single worker with a patent infection originated (colony 1) plus a second, randomly chosen, colony (colony 2) were scanned molecularly. In contrast to visual inspection, molecular analyses revealed significant effects for both the age of animals when the inocula were administered as well as for the source of spores (Table 1).

Within the treatment group that received *terrestris* spores the likelihood of becoming infected was significantly higher for animals at 2 days of age (77·78%) as compared to individuals at 10 days of age (30·00%) (Fisher's exact $P=0{\cdot}004$; differences between colonies within age groups were non-significant: Fisher's exact – 2 days: $P=1{\cdot}000$; 10 days, $P=0{\cdot}141$). Intraspecific inoculations were significantly more successful than interspecific inoculations at causing infections in 2-day-old bees

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Table 2. Numbers of infected and non-infected individuals as determined by dissections and visual detection of spores 21 days after inoculation with *Nosema bombi*

Treatment group (age 2 days) spore source; treatment type	Colony A		Colony B		Total	
	healthy	infected	healthy	infected	healthy	infected
terrestris; trickle* $5 \times (10^5$ spores)	10	0	7	1	17	1
lucorum; trickle* $5 \times (10^5$ spores)	7	2	9	1	16	3
lucorum; single 5×10^5 spores	6	4	6	4	12	8

* One animal in colony A and 2 animals in colony B died prematurely, and were excluded.

(*terrestris* spores = 77·78% infection success *vs* *lucorum* spores = 25·00%; Fisher's exact $P = 0{\cdot}003$; there was no difference between colonies for the *lucorum* spore source: Fisher's exact $P = 1{\cdot}000$).

Experiment 2

Twelve out of 57 individuals exhibited patent infections (3 individuals died prematurely and were excluded from the analyses; no signs of infection were noted in these animals at dissections; Table 2). As in Exp. 1, no spores were detected in faecal samples at 10 days p.i.

Although administration of a single dosage of *lucorum* spores led more frequently to an infection (40·00%) than a trickle dosage (15·79%), the difference was not significant (Fisher's exact $P = 0{\cdot}155$; differences between colonies were non-significant both within trickle- and single-treatment: Fisher's exact – trickle dose: $P = 0{\cdot}582$; single dose: $P = 1{\cdot}000$). Similarly, there was no significant difference between single- and trickle-infected animals in infection intensity (2-ANOVA: Dose-type, $F_{12,1} = 0{\cdot}339$, $P = 0{\cdot}577$; Colony, $F_{12,1} = 0{\cdot}419$, $P = 0{\cdot}535$; Dose-type × Colony, $F_{12,1} = 2{\cdot}260$, $P = 0{\cdot}171$), although infections appeared less intense in bees that had received a single inoculum (Fig. 1).

In contrast to the molecularly identified non-patent infections in Exp. 1, spore origin did not influence the percentage of patent infections resulting from the trickle dose (*terrestris* = 5·56%, *lucorum* = 15·79%; Fisher's exact: $P = 0{\cdot}604$; no significant differences between colonies $P = 0{\cdot}444$). The single individual that was found to be infected in the *terrestris* trickle dose treatment had an infection intensity of ~2·9 million spores which was not significantly different from the intensities recorded in animals treated with a trickle dose of *lucorum* spores (one-sample *t*-test, $t_2 = 2{\cdot}619$, $P = 0{\cdot}12$).

Overall (spanning both experiments), there was a clear effect of dose on the likelihood of developing a patent infection. Among bees that were treated with a single dosage of *lucorum* spores, a 5-fold higher dosage (500 000 spores compared to 100 000 spores) increased the average infection success from 2% (1/50 bees – Exp. 1) to 40% (8/20 bees – Exp. 2)

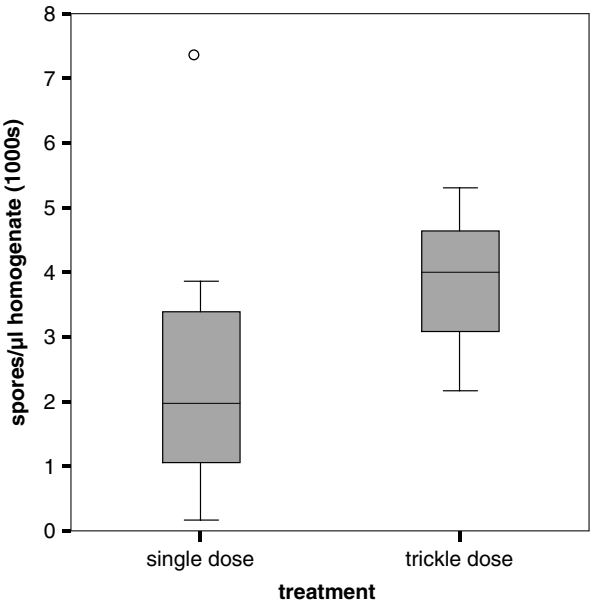

Fig. 1. Differences in *Nosema bombi* spore counts (based on 1 ml dilution of homogenate) between animals infected by single and trickle *lucorum* spore dosage. Data points are boxplots, showing the median value with the box marking the interquartile range; numbers above bars indicate the sample size in each colony; the circle above the bar indicates an outlier.

(Fisher's exact: $P \leqslant 0{\cdot}001$). Nevertheless, the infection intensity in the single bee inoculated by the low dose was not significantly different from the intensity of infection in bees inoculated with a 5-fold higher dose (one-sample *t*-test, $t_7 = 0{\cdot}862$, $P = 0{\cdot}417$).

DISCUSSION

Dose, host-age and donor identity all significantly influence the likelihood of horizontal transmission of *Nosema bombi* to adult bumble bee workers. However, our results also suggest that only host-age and dose are likely to play a role in the evolutionary epidemiology of this microsporidian parasite.

Infection success can be measured in two ways. One, has the parasite established itself in its host? And two, is the parasite capable of transmitting from its newly infected host? Previous studies of *N. bombi* in bumble bees have not distinguished between these

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measures of success (Eijnde and Vette, 1993; McIvor and Malone, 1995; Schmid-Hempel and Loosli, 1998). In our experiments, a 5-fold increase in dosage from 100 000 to 500 000 spores resulted in a 20-fold increase in the prevalence of patent infections. Similar observations have been made for *N. apis* infections in the honey bee (Fries, 1988; Malone *et al.* 2001). Coupled with the absence of patent infections below a dose of 100 000 spores, these results suggest that a minimum dosage in the range of 100 000 spores has to be encountered by an animal to make possible the establishment of a transmittable infection. The intensity of infection in adult-infected bees, at around 4000 spores/μl of abdominal homogenate (regardless of dosage), was of the same magnitude as the majority of infections in a previous study (Schmid-Hempel and Loosli, 1998) but 1 or 2 orders of magnitude lower than in larval-infected bees (Rutrecht and Brown, unpublished data). This suggests that, whilst adult bees can indeed pick up *N. bombi* and develop patent infections, such infections are likely to play a minor role in the epidemiology of the parasite. This is because bees infected as adults should contribute less to the spread of infective spores due to their lower spore dose, combined with the existence of a threshold dosage for further infections.

In contrast to these results, molecular analyses revealed the presence of non-patent infections in 25% of animals inoculated with 100 000 spores. The molecularly recorded prevalences in this study are similar to results from a previous study by Schmid-Hempel and Loosli (1998) who used a similar dosage to induce infections (60 000 spores) and scored infection success via microscopical examination of abdominal homogenate, rather than analysis of faecal samples. In contrast to Schmid-Hempel and Loosli (1998) we found no effects of colony on the likelihood of a bee developing a non-patent infection. However, there was a significant effect of host age, with 2-day-old bees being twice as susceptible as their 10-day-old sisters. In addition, the only patent infection after the 100 000 spore inoculum was found in a 2-day-old bee. This decrease in susceptibility with increasing age is unlikely to be due to changes in host immunity, as immunocompetence decreases over the same time period (Doums *et al.* 2002), but may be due to changes in gut structure as the bee ages.

Obviously, any effects of host-age on infection need to be examined in the context of the host's life-history. In the field, the average life-expectancy of a bumble bee worker is about 20–30 days (Rodd *et al.* 1980; Goldblatt and Fell, 1987; Schmid-Hempel and Heeb, 1991). If it takes 21 days for an infection to become transmittable (Schmid-Hempel and Loosli, 1998) then there is unlikely to be selection on the parasite to successfully infect older bees. In contrast, bees infected in the first few days after eclosion have a much higher probability of surviving to transmit spores. Thus this age effect, combined with previous studies (Eijnde and Vette, 1993), suggests that *N. bombi* has evolved to infect larvae and young adults preferentially because such infections are significantly more likely to lead to further spread of the parasite.

Intraspecific inoculations were three-times more likely to result in non-patent infections. This is surprising, given that recent genetic analyses have demonstrated that *N. bombi* has no host-species related population structure, which should be indicative of the absence of species-specific interactions (Tay *et al.* 2005). Schmid-Hempel and Loosli (1998) conducted the obverse of our experiment, inoculating 3 different host species (*hypnorum*, *lapidarius* and *terrestris*) with 1 inoculum. A re-analysis (binary logistic regression with colony and species entered as predictor variables) of their data found no significant effects of host species on parasite establishment (the final model contained colony, but not species, as a predictor variable for establishment: 84·4% cases categorised correctly, Colony: Wald-statistic = 26·03). Furthermore, in contrast to our non-patent infections, there was no effect of intra- versus interspecific inoculations on the likelihood of developing patent infections in Exp. 2. If anything, the trend was in the opposite direction. Thus, while our experiments have revealed intriguing evidence for species-specific interactions in this single-parasite/multiple-host species system, our data on patent infections are in line with molecular work that suggests *N. bombi* is a generalist parasite of multiple bumble bee host species (Tay *et al.* 2005).

In conclusion, our results, in combination with previous studies (Eijnde and Vette, 1993; Schmid-Hempel and Loosli, 1998), demonstrate that the epidemiology of *N. bombi* depends upon successfully infecting young adults and larval bees. The mechanisms that underlie such age-dependent infection success remain unexplored in microsporidia in general. Newly eclosed bees remain within the nest for the first few days of their adult life, and combined with the positive relationship between dose and infection success this suggests that the colony, where spores accumulate and are protected from destructive UV, is likely to be the major arena for worker infection. Thus the relatively low level of horizontal transmission indicated by our results, either among or within colonies, may suggest low levels of virulence in this microsporidian parasite. Further studies of the epidemiology of *N. bombi* should concentrate on within-colony epidemiology and the route and frequency of cross-colony transmission.

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