Within colony dynamics of Nosema bombi infections: disease establishment, epidemiology and potential vertical transmission

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Within colony dynamics of *Nosema bombi* infections: disease establishment, epidemiology and potential vertical transmission*

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Abstract – Successful growth and transmission is a prerequisite for a parasite to maintain itself in its host population. *Nosema bombi* is a ubiquitous and damaging parasite of bumble bees, but little is known about its transmission and epidemiology within bumble bee colonies. The impact of host demography and colony life-cycle on the transmission and reproduction of *N. bombi* were examined in *Bombus lucorum* colonies. Parasite success was highest when infecting colonies where larval exposure to the parasite was high. The later individual bees were born in the colony life-cycle, the higher their infection intensity, but after eclosion individual parasite loads did not increase, indicating either host control of the parasite, a balance between internal infection and the production of transmission stages, or a switch in the parasite's growth strategy after eclosion to the production of transmission stages. Finally, trans-ovarial vertical transmission of *N. bombi* was suggested using molecular probes.

Nosema bombi / *Bombus lucorum* / **transmission** / **epidemiology** / **vertical transmission**

1. INTRODUCTION

Parasites rely on successful host-to-host transmission to maintain themselves in host populations, and the rate, route and mechanism of transmission play a considerable role in determining the impact that they will have on their host. Consequently, understanding parasite epidemiology is important for managing and understanding host-parasite systems. Whilst social bees are host to numerous parasite species (Schmid-Hempel, 1998), few studies have examined the evolutionary epidemiology of parasite infections (Fries and Camazine, 2001). This is particularly true for bumble bees, where epidemiological studies have been almost absent (but see Otterstatter and Thompson, 2007). Given the ecological and economic importance of bumble bees (Goulson, 2003; Velthuis and van Doorn,

2006), their current decline (Goulson, 2003; Fitzpatrick et al., 2007), and the potential role that has been suggested for parasites in driving this decline (Thorp and Shepherd, 2005; Winter et al., 2006), it is important that we investigate the epidemiology of bumble bee parasites.

As annual social insects, bumble bees pose particular problems for parasite transmission and epidemiology. Firstly, the parasite has to find, infect and multiply within an individual. Secondly, it then needs to spread within the colony prior to the death of its original host. Thirdly, this infection within the colony needs to reach sufficiently high levels to result in the infection of new gynes, who will carry the parasite into the next annual generation. Fourthly, because bumble bee colonies show considerable variation in reproductive performance, even under ad libitum conditions (Müller and Schmid-Hempel, 1992a, b), and because parasites themselves reduce the likelihood of a colony producing gynes (Brown et al., 2003;

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Rutrecht and Brown, in review), a final critical step in the parasite's life history is its successful transmission to and subsequent establishment in new colonies (see steps 1–3 above).

Nosema bombi Fantham & Porter is a common microsporidian parasite of bumble bee species (Tay et al., 2005). While previous studies have presented a contradictory picture of the impact of this parasite (Fantham and Porter, 1914; Betts, 1920; De Jonghe, 1986; Fisher and Pomeroy, 1989; Shykoff and Schmid-Hempel, 1991; van den Eijnde and Vette, 1993; McIvor and Malone, 1995; Macfarlane et al., 1995; Schmid-Hempel and Loosli, 1998; Imhoof and Schmid-Hempel, 1999; Whittington and Winston, 2003), recent controlled colony-infection studies suggest that it can have a significant negative impact on bumble bee colony growth and reproduction (Otti and Schmid-Hempel, 2007; Rutrecht and Brown, unpubl. data). *N. bombi* is believed to rely on horizontal transmission between individuals (McIvor and Malone, 1995; Fries et al., 2001), and studies of the epidemiology of this system suggest that both workers (Schmid-Hempel and Loosli, 1998) and larvae (van den Eijnde and Vette, 1993) can be infected, but that larval infections are most likely to result in further transmission (Rutrecht et al., 2007). Colonies pick up the parasite from the environment (Imhoof and Schmid-Hempel, 1999) suggesting that it is transferred between colonies by infected workers contaminating shared food sources such as pollen or nectar at flowers, as has been shown for *Crithidia bombi* Lipa and Triggiani another microparasite of bumble bees (Durrer and Schmid-Hempel, 1994). However, nothing is known of the dynamics of transmission within colonies, or what factors determine how likely the parasite is to spread sufficiently to enable infection of new gynes or further transmission between colonies.

In this study we used controlled colony infections to determine how demography impacts on the epidemiology and spread of *N. bombi* infections within colonies. Specifically, we asked whether larval exposure determines infection success at the colony level, whether infections build up over the life of the colony or of individual animals, and finally whether

transovarian vertical transmission from mother to offspring exists for this parasite.

2. MATERIALS AND METHODS

2.1. Experimental colonies

Twelve *B. lucorum* colonies were reared and successfully infected with *N. bombi* under controlled laboratory conditions. All colonies were founded by *B. lucorum* spring queens, collected in the vicinity of Dublin, Ireland in 2004.

Following capture queens were established in the laboratory in perspex nesting boxes (17 × 10 × 6 cm) supplied with sugar water (diluted Apiinvert®) and pollen (Hortico Ltd. Ireland) ad libitum under standard rearing conditions (28 °C, 50% relative humidity). Successful rearing of control colonies (see below) on the same resources indicates that they were not contaminated with *N. bombi* spores. After queens had established nests and at least 4 workers had hatched, the comb and bees were transferred to bigger observation hives (adapted from Pomeroy and Plowright, 1980). For their entire life cycle bees had no access to the field but could "forage" in wooden feeding boxes (11 × 13 × 21 cm) provided for each colony. These boxes were connected to the hive via a tube and were supplied on a daily basis with thawed fresh pollen and sugar water. As soon as the fifth worker had hatched colonies were presented with a standardised inoculum. The inoculum that was used for all the infections was prepared from a mixture of two different spore isolates taken from two infected *B. lucorum* spring queens (also caught in the vicinity of Dublin in 2004), and stored in aliquots at −80 °C until use in treatments. The inoculum was administered in the form of 20 μL of spore suspension containing 2.5×10^4 spores per μL dispensed onto a pollen pellet – this treatment mimics either early stage transmission via flowers in the field through collection of contaminated pollen (Imhoof and Schmid-Hempel, 1999) or colony-founding by a *N. bombi* infected spring queen via contamination of food within the nest. The choice of dose was based on dosage levels which had led to successful experimental infection of individuals in previous studies (McIvor and Malone, 1995; Schmid-Hempel and Loosli, 1998). Pollen pellets were deposited in colonies twice a week for five weeks (ten treatments for a total of 5×10^6 spores); other pollen food sources were removed until each treated pellet was consumed. All experimental colonies were

successfully infected (15 control colonies which were raised with un-treated pollen pellets showed no signs of infection).

Colony development was recorded on a daily basis. Dead individuals were removed and dissected immediately, or later from frozen. For colonies reaching worker numbers of above 30 a subset of individuals was dissected. Subsets covered the whole period over which animals eclosed, and consisted of a minimum of 30 workers; not every animal could be reliably examined for infection due to cases of decomposition. Males were removed from colonies at three days of age, which mimics the course of natural events (Alford, 1975), transferred to separate boxes and either kept until natural death or allocated to the male longevity experiment (see below). Again, similar to worker samples, natural death male dissection samples consisted of a stratified subset.

The intensity of *N. bombi* infections in abdominal tissue was quantified by homogenising abdomens 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. The resulting spore suspension was then diluted by half and counted in a haemocytometer (Neubauer chamber) under the microscope at magnification ×400. While *Nosema* infections may eventually spread to all host tissues including the brain, abdominal tissue rather than whole specimens were used in our analysis as abdominal infections are more likely to represent infections that are transmittable or contribute to nest contamination, i.e it is unlikely that infection of nervous tissue will be further transmitted.

2.2. Infection dynamics and colony demography

Previous work (van den Eijnde and Vette, 1993; Rutrecht et al., 2007) suggested that horizontal transmission of *N. bombi* is most likely to occur at the larval stage of the host. To determine whether larval exposure to the parasite plays a role in within colony epidemiology, we measured the percentage of larvae that were exposed to the parasite and compared it to prevalence and intensity of infection in adult animals. The average developmental period from egg to eclosion for workers of *Bombus terrestris*, a species closely related to *B. lucorum*, is approximately 31 days: after a seven-day egg stage, larvae are continuously fed through a larval period of about 14 days until the pupal stage is entered, which lasts about ten days (van der Steen and Donders, unpubl. data). Larvae are progressively fed on pollen and nectar (sugar water), which is regurgitated by the nurse bumble bees (Alford, 1975). Consequently, for each worker we determined whether it had been exposed as a larva to *N. bombi* spores, based on whether it eclosed on the 11th day or later after the first introduction of *N. bombi* spores to the colony. Animals which eclosed prior to this time were unexposed at the larval stage.

2.3. The effect of age and time on infection intensity

To measure how parasitaemia builds up within a colony over time, as well as the impact of animal age on individual infections, we conducted an experiment with males from 5 colonies. After removal from the nest at three days of age (see above) a subset of males were sequentially allocated to be culled at 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 days of age; i.e. the first male born during the experiment was culled at 3 days of age, male number 10 was culled at 21 days of age and male number 11, again, was culled at 3 days of age, etc. Animals that died a natural death prematurely to their allotted lifespan category were discarded from the experiment (however, these animals were then used for dissections to help determine male prevalence). After being culled, males were stored frozen prior to assessing their infection status and the intensity of infection, if present. Infection dynamics were assessed in males and not in workers (which would have been equally interesting) as the removal of workers from the colony would have impacted overall colony development.

## 2.4. Potential for vertical, transovarial transmission

To assess whether *N. bombi* can be vertically transmitted, we used three egg-laying workers [worker oviposition occurs in bumble bee colonies after the queen has lost her dominance (Röseler and van Honk, 1981; van Honk et al., 1981)] that carried high levels of infection (heavy infections can

be visually distinguished by a lobate and porcelain-white appearance of the fatbody tissue which is the result of the accumulation of large masses of spores). The ovaries of these bees were dissected out under the binocular microscope and repeatedly washed in petri dishes filled with fresh deionised water (dH$_2$O). A minimum of six well-developed eggs were then dissected from these ovaries and again washed five times in fresh dH$_2$O. A single egg of each batch (originating from the same worker ovary) was then examined under the microscope at ×400 for any evidence of adhering spores; all examinations were negative. The whole batch of eggs was then stored in 200 μL of dH$_2$O in the freezer at −80 °C until molecular analyses. Eggs that had been laid by one of the workers and a larva at a late developmental stage (just before pupation) were dissected out of their respective brood cells, washed repeatedly as described above, and frozen separately for later molecular analyses. Molecular analyses were conducted following a protocol developed by Klee et al. (2006).

3. RESULTS

3.1. Impact of larval exposure on prevalence and intensity of infection

There was a significant positive correlation between the percentage of worker larvae exposed to *N. bombi* spores and the percentage of workers that became infected by the parasite (Pearson product-moment correlation coefficient (PMCC), $N = 13$, $r^2 = 0.348$, $P = 0.034$; Fig. 1a). Similarly, mean intensity of infection across workers in a colony also increased with the percentage of larvae exposed to spores (PMCC, $N = 10$, $r^2 = 0.772$, $P = 0.001$; Fig. 1b). To explore whether differences among colonies in prevalence of infection were driven by colony-specific factors (e.g., genotype or maternal effects) or larval exposure we used a binary logistic regression with colony and larval exposure as predictor variables and infection by *N. bombi* as the dependent variable. In a model where the interaction term was also presented to the model, 88% of animals were assigned correctly to infection status, but neither of the terms included in the model were individually significant (colony: Wald-statistic = 6.418, df = 8,
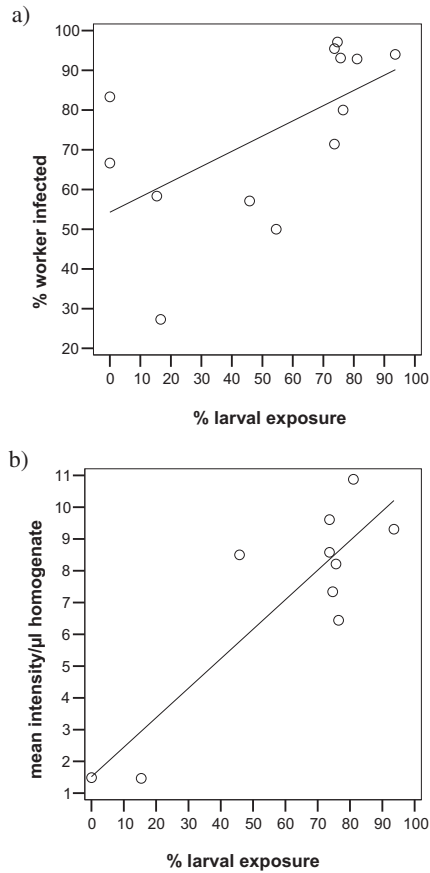
Figure 1. The relationship between larval exposure and levels of (a) infection prevalence, and (b) intensity in workers. Individual circles represent colonies and the line is the line of best fit.

$P = 0.601$; colony × larval exposure: Wald-statistic = 4.078, df = 8, $P = 0.085$). Excluding the interaction term resulted in a model containing both colony and larval exposure as significant predictive variables, correctly classifying 83.7% of animals (larval exposure: Wald-statistic = 7.448, df = 1, $P = 0.006$, Exp (B) = 3.675; colony: Wald-statistic = 23.271, df = 8, $P = 0.003$).

In contrast to workers, neither the prevalence nor the mean intensity of infection in males correlated significantly with the % of worker larvae exposed to parasite spores (Spearman's rank correlation coefficient (SRCC), $N = 12$, $r_s = 0.538$, $P = 0.071$; SRCC, $N = 7$, $r_s = -0.492$, $P = 0.263$,
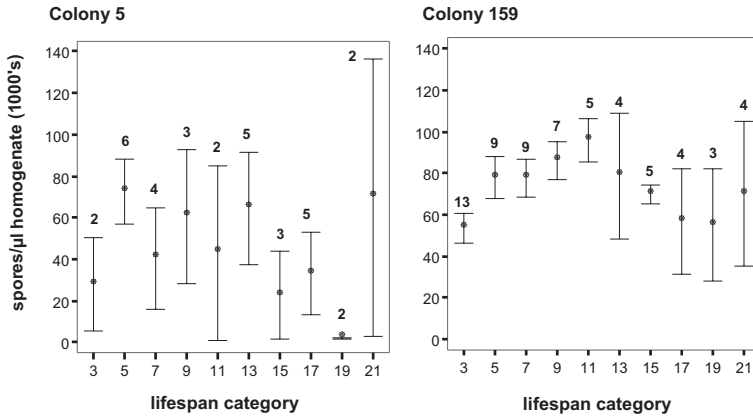
Figure 2. Differences in mean infection intensity of lifespan categories for males from colony 5 and 159. The x-axis shows the different lifespan categories in days. The y-axis shows the amount of spores in 1 μL homogenate (based on 1 mL dilution of the original homogenate). Data points are mean values ± standard error bars, adjacent numbers show the sample size for each lifespan category.

respectively). However, there were significant positive relationships between prevalence and mean intensity of infection in males and parasite prevalence in workers (SRCC, $N = 12$. $r_s = 0.812$, $P = 0.001$; SRCC, $N = 10$, $r_s = 0.818$, $P = 0.004$, respectively).

3.2. Male infection dynamics

Of the 5 colonies, only 2 provided sufficient males to have replicates at each time point. There was no significant effect of the time a male was allowed to live post-hatching and the average intensity of infection (colony 5, data non-transformable: Kruskal-Wallis $\chi^2 = 5.760$, df $= 9$, $P = 0.764$; colony 159: ANCOVA $F_{9,63} = 1.675$, $P = 0.119$; Fig. 2). In other words, individuals that were culled shortly after eclosion did not on average carry lower or higher spore loads than animals that were culled at a later date. However, there was a significant effect of the birth date of males (ANCOVA $F_{1,63} = 27.736$, $P < 0.001$), with males carrying higher parasite loads as the colony aged.

Given the absence of an effect of individual lifespan on intensity of infection, data from all 5 colonies were analyzed to examine the relationship between colony age and male parasite loads. However, while 4 out of 5 colonies exhibited a positive correlation between colony age and male parasite load, it was only statistically significant for colony 159 (colony 159: $r^2 = 0.282$, $P < 0.001$; colony 168: $r^2 = 0.582$, $P = 0.078$; colony 161: $r^2 = 0.232$, $P = 0.134$; colony 5: $r^2 = 0.007$, $P = 0.649$; colony 172: $r^2 = 0.137$, $P = 0.413$; Fig. 3). Interestingly, the two colonies where the effect was absent or weakest (colonies 5 and 172) had lower percentages of workers exposed to the parasite at the larval stage than did the remaining three colonies (Tab. I) and consequently lower prevalence of the parasite in both workers and males (Tab. I). These results suggest that high parasite prevalence goes hand-in-hand with increasingly intense infections.

3.3. Male vs. worker infection intensity

There was no effect of sex on infection intensity ($F_{1,75} = 0.402$, $P = 0.551$). Similarly, there was no effect of colony of origin ($F_{5,75} = 2.392$, $P = 0.180$) nor the interaction between sex and colony ($F_{1,75} = 0.420$, $P = 0.833$). Thus, irrespective of their maternal background, males and workers carried similarly intense infections of *N. bombi* (Fig. 4). Across all animals (males and workers) the absolute number of spores per bee abdomen ranged from $3.7 \times 10^5 - 4 \times 10^8$ spores.
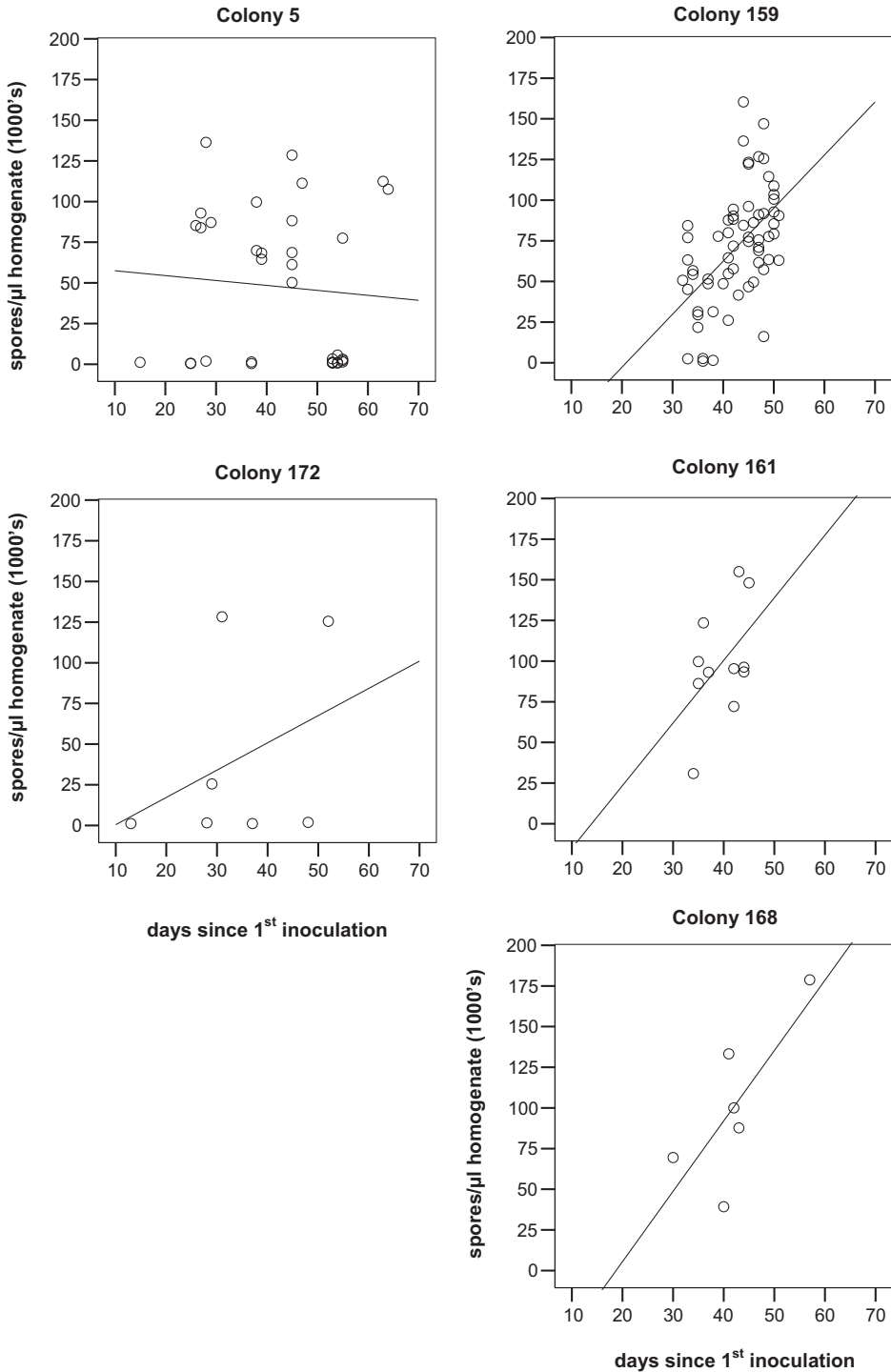
Figure 3. Regression of infection levels of males against the day males were born (day of eclosion since the start of colony infections). Circles represent individual males. See text for statistics.

Table I. Prevalence of infection for males and workers and % of workers exposed to *N. bombi spores* as larvae in five colonies.

	Colony				
	5	159	161	168	172
% males infected	79.36	97.47	96.67	100	45.83
% workers infected	57.10	92.85	95.45	94.00	58.34
% workers with larval exposure	45.84	81.05	76.68	93.59	15.38
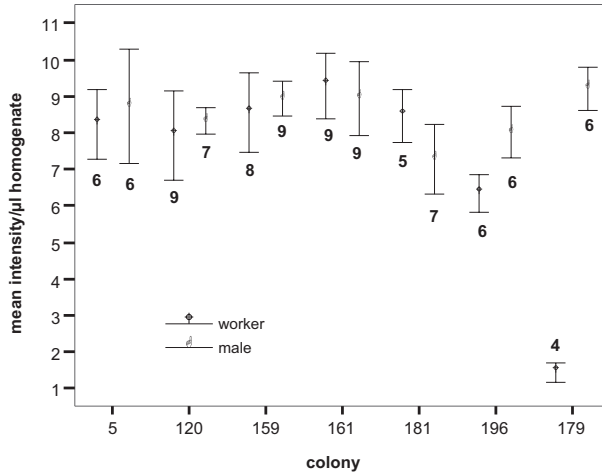
Figure 4. Differences in mean infection intensities for workers and males. The x-axis shows the different colonies from which individuals originated. The y-axis shows the mean infection intensity per µL homogenate (1000's) (based on 1 mL dilution of the original homogenate); square-root transformed data. Data points are mean values ± standard error bars; numbers below and above bars show the sample size in each category.

3.4. Evidence for vertical transmission

Three samples consisting of five eggs each which were dissected from the ovaries of individual worker bees and one sample of laid eggs, as well as one larva produced by the same worker, were molecularly typed. *N. bombi* was detected unambiguously in every egg sample as well as in the larva (Fig. 5).

4. DISCUSSION

While social insects are exploited by numerous parasites, we know remarkably little about how parasites establish themselves in host colonies, their subsequent epidemiology and how this relates to their maintenance within the host population at large (Schmid-Hempel, 1998). Here we have shown that host demography at the time of infection is central to the establishment and epidemiology of *Nosema bombi* within bumble bee colonies. We also demonstrate, for the first time, the potential for transovarial vertical transmission of this parasite, which may play an important role in maintaining the parasite population within hosts.

Our results show that the establishment and spread of *N. bombi* infections in bumble bee colonies depends on the proportion of workers that are exposed to parasite spores during larval development. Previous studies of *N. bombi* have suggested that infection of individual larvae is considerably easier than infection of adult workers (van den Eijnde and Vette, 1993;
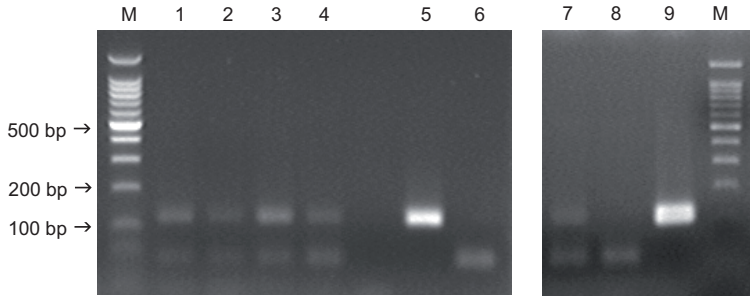
Figure 5. PCR amplicons of *N. bombi*, amplified with the primer pair ITS-f2/r2 (expected amplicon length 118 or 122 bp), resolved in 2% agarose gel and stained with ethidium bromide. Lane M, 100bp DNA marker; lane 1, worker laid eggs; lane 2,3 and 4, eggs dissected from worker ovaries; lane 5 and 9, purified *N. bombi*; lane 6 and 8, negative; lane 7, worker-produced male larva.

Rutrecht et al., 2007; but see Schmid-Hempel and Loosli, 1998). Here we have shown that in the social context, larval infection plays a key role in the spread of the parasite to the worker population. Furthermore, the intensity of infections also increased as larval exposure increased. This suggests that, even if infection of adult workers occurs, it results in infections that are considerably less well-developed than infections via larvae. While male infection prevalence and intensity did not correlate with the proportion of workers who were exposed to the parasite as larvae, they did correlate with worker prevalence. These results together suggest a model for the establishment and epidemiology of *N. bombi* infections within and between naive bumble bee colonies. First, worker bees contact spores that have been deposited at food sources by other infected bumble bees [of any caste or species – all bumble bee spp. analysed to date suffer from the same microsporidian, *Nosema bombi* (Tay et al., 2005)] while foraging. While workers may become infected themselves from such contact (Schmid-Hempel and Loosli, 1998) they are unlikely to be able to spread the parasite as a result of such infections due to the long lag-time before patent (transmittable) infections appear (Rutrecht et al., 2007). However, workers will bring the contaminated pollen back to the nest where it is fed, or 'vectored' to the larval brood. Second, individuals (including all castes) that consume the parasite at the larval stage (and above a threshold dosage; Rutrecht et al., 2007) will develop intense in-

fections and spread the pathogen via faeces soon after eclosure. Three, the resulting accumulation of infective material in the nest will increase the overall force of infection to new larvae, enabling the parasite to establish itself within the colony, as evidenced by the increase in intensity of infection seen with increasing larval exposure in workers and across the colony cycle in males. Finally, larvally-infected workers will spread the parasite in the foraging environment, where it can encounter naive workers from different colonies. The earlier in the colony life-cycle the initial infection takes place, the greater the probability of transmission of the parasite both within and between colonies. Furthermore, because males can transmit the infection during mating (Otti and Schmid-Hempel, 2007; Rutrecht and Brown, in review), and because male infection rates increased with increasing prevalence in workers, which in turn was determined by larval exposure, early infections will also result in more horizontal transmission of the parasite from males to new queens.

Interestingly, in addition to larval exposure, colony identity was a significant predictor of infection prevalence within colonies. A similar effect has been seen in controlled individual inoculations of adults (Schmid-Hempel and Loosli, 1998; but see Rutrecht et al., 2007). Such colony-level patterns in susceptibility may be explained by differences in genetic background (e.g., in the immune response) or differences among colonies in their hive environment. In the absence of breeding

experiments, it is currently impossible to distinguish between these explanations. In contrast, while colony background predicted the likelihood of infection taking place, it did not affect the actual intensity of infections in infected animals.

How does infection intensity vary across the life of an individual bee? Our results suggest that, once a male has matured (i.e., gained its adult coat colour and hardened its wings so that it can leave the colony) it already has its maximum infection intensity or parasite load. Consequently, if spore production carries on in these animals it must be balanced by the amount of infective spores shed in the faeces. It is intriguing that this same pattern is seen across almost 100-fold differences in intensity. One explanation for this is that the host might be able to regulate the parasite population after it has matured, perhaps due to changes in immunocompetence, preventing low level infections from developing further. Alternatively, within-host parasite growth may be limited to certain parts of the host life-cycle, in a similar way to changes in infectivity (van den Eijnde and Vette, 1993; Rutrecht et al., 2007), with a switch from internal growth to the production of transmission stages, and thus initial infection dose might determine final infection intensity. It would be interesting to test this by conducting controlled dosage experiments on larvae at different stages of development.

O'Donnell and Beshers (2004) suggested that male social insects should be particularly susceptible to parasites, and that this could have played a role in the evolution of eusocial systems. Previous studies have shown that workers have stronger immune systems than males (Moret and Schmid-Hempel, 2001; Gerloff et al., 2003; Baer and Schmid-Hempel, 2006). However, we found no difference in the level of infection intensity between males and workers, and prevalence levels were, if anything, higher in males. Similarly, Schmid-Hempel and Loosli (1998) found no difference in susceptibility between worker and male larvae to controlled infection by *N. bombi*. Together with previous studies (Ruiz-González and Brown, 2006) this suggests that the assumption underlying the model of O'Donnell

and Beshers (2004) of haploid susceptibility is unfounded, at least in bumble bees.

N. bombi has previously been believed to be transmitted only horizontally at the level of individual infections (McIvor and Malone, 1995; Fries et al., 2001) (although at the colony-level, vertical transmission occurs via hibernating queens between generations). However, these previous studies were limited by either technique [visual identification of spores by light microscopy (McIvor and Malone, 1995), which is insensitive to light infections (Rinder et al., 1998)] or sample size (one queen; Fries et al., 2001). Here we provide the first evidence that *N. bombi* may be transovarially transmitted, at least in reproducing workers. While novel, this discovery corresponds well with the existence of transovarial transmission in other species of *Nosema* (Raina et al., 1995; Wittner and Weiss, 1999; Dunn et al., 2000; Dunn and Smith, 2001) and reports of severe ovarian infections in the closely related species *N. apis* (Steche, 1960). Clearly, further studies on the rate of transmission from infected queens and workers to their offspring are required. However, the existence of vertical transmission may play a significant role in maintaining this parasite in its host populations, as well as having significant implications for the evolution of virulence in this system (Herre, 1993; Lipsitch et al., 1996).

Overall, our study showed that inter-colony variation in colony development in conjunction with the timing of parasite contact can have a major influence on disease establishment, the likelihood of successful further transmission, and thus maintenance of the parasite in the host population. Together with the discovery of a potentially novel route of transmission, and other recent studies (Otti and Schmid-Hempel, 2007; Rutrecht et al., 2007), we are close to unravelling the interaction between this previously enigmatic parasite and its bumble bee hosts.

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Dynamique des infections à *Nosema bombi* à l'intérieur de la colonie : mise en place de la maladie, épidémiologie et transmission verticale.

***Nosema bombi* / *Bombus lucrorum* / transmission verticale / épidémiologie / parasite / microsporidie**

Zusammenfassung – Dynamik von *Nosema bombi* Infektionen in Hummelvölkern: Etablierung der Krankheit, Epidemiologie und Möglichkeit der vertikalen Übertragung. Zum Verständnis der Bedeutung eines Parasiten ist es wichtig, die Dynamik seiner Übertragung im Wirt zu kennen. Das Mikrosporidium *Nosema bombi* parasitiert Hummeln und führt nicht nur zur Behinderung einzelner Tiere, sondern kann den Fortpflanzungserfolg ganzer Hummelvölker beeinflussen. Wir untersuchten die Übertragung dieses Parasiten in Laborvölkern von *Bombus lucorum*, indem wir Kolonien kontrolliert inokulierten und im Anschuss daran untersuchten, wieviele Einzeltiere infiziert waren und in welchem Ausmass sich diese Infektionen auf den Lebenszyklus der Kolonie auswirkten. Mittels molekularer Methoden untersuchten wir ausserdem, ob es zu einer vertikalen Übertragung des Parasiten vom Ei auf die Nachkommen kam. Parasiteninfektionen waren am erfolgreichsten, wenn eine grosse Zahl an Bienenlarven in Kontakt mit dem Parasiten kamen. Ausserdem nahm die Intensität der Infektionen im Verlauf des Lebenszyklus der Kolonien zu. Die Parasitenbelastung einzelner Tiere stieg jedoch nach dem Schlüpfen nicht weiter an. Mittels molekularer Methoden konnten wir zeigen, dass *N. bombi* Sporen bereits in Eiern zu finden sind, die von Arbeiterinnen abgelegt worden waren. Dies deutet darauf hin, dass der Parasit von einer Generation auf die nächste vertikal übertragen werden kann, ohne dass eine orale Infektion erforderlich ist. Unsere Ergebnisse tragen damit signifikant zum Verständnis dieses wichtigen Hummelparasiten bei.

***Nosema bombi* / *Bombus lucorum* / Übertragung / Epidemiologie / vertikale Übertragung**

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