

## A scientific note on *Nosema bombi* infection intensity among different castes within a *Bombus auricomus* nest

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Bumble bees (*Bombus*) provide vital services to agricultural and natural plant systems (Kremen et al. 2002; Fontaine et al. 2006). A survey across diverse North American *Bombus* species detected higher prevalence of infection by the microsporidian parasite *Nosema bombi* in declining relative to non-declining species, indicating a correlation between *Nosema* and *Bombus* population declines (Cameron et al. 2011). Although it has been shown in the laboratory that *N. bombi* infection is detrimental to the fitness of *Bombus terrestris* and *Bombus lucorum* (Otti and Schmid-Hempel 2007; Rutrecht and Brown 2009), much remains unclear regarding *N. bombi*'s ecology and how it interacts with *Bombus* in the field.

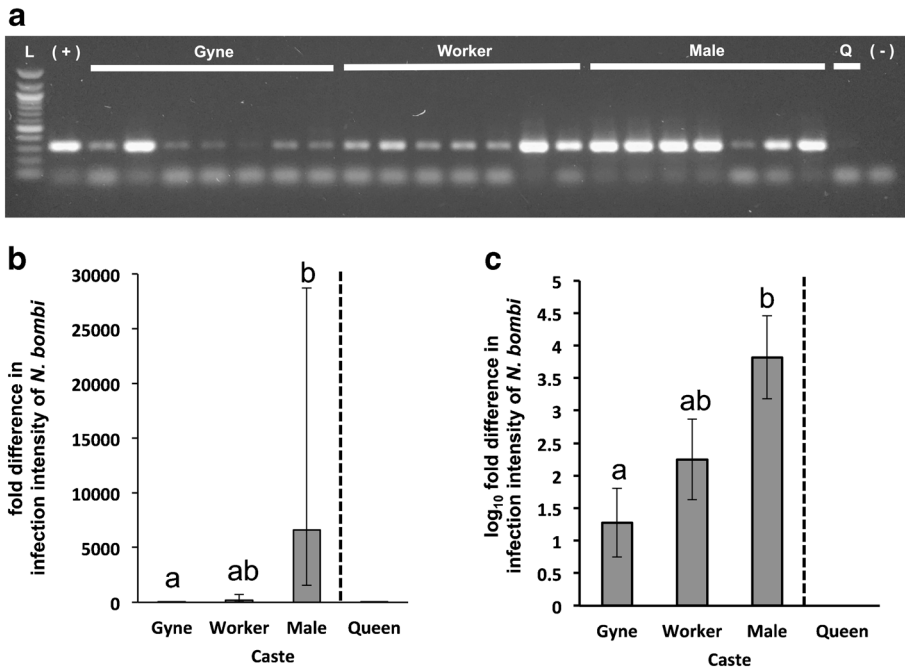
Previous field studies have suggested that *Nosema* is more prevalent or abundant in males than in females of some *Bombus* species (Shykoff and Schmid-Hempel 1991; Gillespie 2010; Huth-Schwarz et al. 2012). In a laboratory study, however, contrasting results were found (Rutrecht and Brown 2008). It is possible that genetic differences among *Bombus* species/*Nosema* strains, geographic distribution, variability in timing of infection, and within-colony epidemiology (Rutrecht and Brown 2008) may lead to different host-parasite interactions and *Nosema* infection patterns. Given the association between high *Nosema* prevalence and specific *Bombus* taxa

undergoing population declines in North America, it is important to examine the host-parasite interactions in other, less-characterized, North American *Bombus* species.

In a life history study of a *B. auricomus* colony found in Urbana, Illinois (Dean et al. 2015), we detected the presence of *N. bombi* in all of a few individuals tested ( $n = 10$ ). Because these included a sample of bees from each caste (parent and offspring queens (gynes), workers, and males), the colony provided an opportunity for further testing of caste effects on *Nosema* infection intensity in *B. auricomus* while minimizing influences of genetic and environmental variation. Here, we examined whether caste effect exists in *B. auricomus* by comparing *Nosema* infection intensities in 22 individuals, including the founding queen, seven gynes, seven workers, and seven males.

We extracted gut DNA from the bees using previously described methods (Dean et al. 2015). Initial PCR testing using *N. bombi*-specific primers (NbombiSSUJf1 and NbombiSSUJr1) (Klee et al. 2006) with 10 ng DNA templates found that all samples were infected with *N. bombi*. Initially, male samples appeared to exhibit greater amplicon intensity than those of other castes (Figure 1a). To validate this finding, we conducted qPCR on the samples using *N. bombi*-specific primers (BOMBICAR) (Plischuk et al. 2009), as well as primers targeting a *Bombus* arginine kinase (AK) gene fragment (Barribeau and Schmid-Hempel 2013). qPCR was conducted using the GoTaq qPCR Master Mix (Promega, Madison, WI) and a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA), using standard conditions (annealing at 58 °C). Three technical

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**Figure 1.** *Nosema* infection in different *B. auricomus* castes. **a** Diagnostic PCR detection of *N. bombi*. L, 100-bp ladder; (+), positive control, a verified *N. bombi*-infected *B. auricomus* worker; Q, parent queen; (-), negative control. **b**, **c** qPCR results showing different *N. bombi* infection intensity in different castes. The data were transformed to fold (**b**) or log<sub>10</sub> fold (**c**) difference in infection intensity for figure illustration. Different letters indicate significant differences.

replicates were tested for all samples (24 ng DNA template). The PCR efficiencies were 90–100 % ( $R^2 > 0.99$ ) and melting curves showed that only the correct amplicons were produced. The data were analyzed using the  $2^{-\Delta\Delta C_T}$  method (Livak and Schmittgen 2001). The *N. bombi* abundance ( $C_T$ ) was normalized against the *Bombus* AK gene (to obtain  $\Delta C_T$ ). To correct for haploid/diploid difference between sexes, we halved the infection intensity in male samples by adding 1 to their  $\Delta C_T$  values. For each sample, the intensity ( $\Delta C_T$ ) relative to that of the queen was calculated ( $\Delta\Delta C_T$ ). The  $\Delta\Delta C_T$  values for the workers, gynes, and males were compared using non-parametric Kruskal-Wallis and post hoc tests (NPTESTS; SPSS, IBM, Armonk, NY).

The results indicate that *N. bombi* infection intensity was caste-dependent in this colony of *B. auricomus* ( $P = 0.027$ ). Males had greater *N. bombi* levels than gynes ( $P = 0.023$ , Bonferroni adjusted), while the workers were intermediate between these two castes (Figure 1b, c). The queen had relatively low *N. bombi* load, similar to that of the gynes (Figure 1b, c). Although it is unclear during which stage of the colony lifecycle the bees were infected, and whether there is an age effect on the data, the fact that both older (queen and

worker) and younger (gyne) females had lower *Nosema* levels than males supports the hypothesis that sex difference is a factor affecting infection intensity.

Caste difference in insects can lead to different physiology, behavior, and gene regulation (Watson et al. 1985; Colgan et al. 2011). Genome ploidy could also influence gene expression or affect pathogen/parasite recognition (Nuismer and Otto 2004; Borges et al. 2012); it is possible that ploidy difference can lead to different susceptibility to *Nosema* across *B. auricomus* sexes/castes. The idea that haploid males may be more susceptible to parasite infection than females (O'Donnell and Beshers 2004) has also been tested in some *Bombus* species using *Crithidia* and *N. bombi* (Ruiz-Gonzalez and Brown 2006; Rutrecht and Brown 2008). In these studies, males and females exhibited no significant difference in infection level. It could be that the within-colony epidemiology of *Nosema* varies with the infection method used (artificial/natural) or is species-dependent, and thus our samples exhibited different infection patterns due to any of these factors. Further investigation is needed to validate the ploidy difference hypothesis.

*Bombus* males can transmit *Nosema* during mating (Rutrecht and Brown 2009). Males usually emerge late in the colony life cycle and leave soon afterward as they

function mostly to mate with gynes outside of the nest; they do not typically return to the colony. Females, on the other hand, comprise the initial worker force and remain in the nest after males have departed, engaging in foraging and the nursing of larvae (Goulson 2003). Females thus have a greater chance of making direct contact with the potentially more susceptible larvae (Van den Eijnde and Vette 1993; Rutrecht and Brown 2008). The gynes are also crucial to colony establishment and may be subject to additional protection (Cremer et al. 2007). Thus, selection for defense against *Nosema* infection may be stronger in females than in males. From the parasite's perspective, the male's early departure from the colony and higher *Nosema* load may facilitate the parasite's dissemination. These hypotheses require further testing. Future examination of similar interactions in other *Bombus* species may help develop approaches contributing to conservation of pollinators in the USA.

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#### Note scientifique sur l'intensité de l'infection par *Nosema bombi* parmi les différentes castes à l'intérieur d'un nid de *Bombus auricomus*

#### Eine wissenschaftliche Notiz zur Infektionsintensität von *Nosema bombi* zwischen verschiedenen Kasten eines *Bombus auricomus* Nestes

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