

NOTES AND COMMENTS



Transportation and pollination service increase abundance and prevalence of *Nosema ceranae* in honey bees (*Apis mellifera*)

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Received 5 July 2013, accepted subject to revision 3 September 2013, accepted for publication 30 October 2013.

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Keywords: *Apis mellifera*, *Nosema ceranae*, transportation, pollination

The Western honey bee (*Apis mellifera* L.) is the most beneficial insect to humans due to the pollination service it provides to agriculture. Honey bees live under many different types of stress. There are external parasites such as *Varroa destructor*, microsporidian pathogens such as *Nosema ceranae* and *N. apis* (Chen and Huang, 2010), and small hive beetles. Aside from parasites and diseases, long distance transportation also affects honey bee physiology (Ahn *et al.*, 2012). There are studies showing that polyfloral pollen is healthier than monofloral pollen for caged honey bees (reviewed by Huang, 2012). However, it is not clear how the use of colonies for a pollination service, which includes transportation, displacement and feeding on one type of pollen, affects honey bee health.

N. ceranae is a wide-spread fungal pathogen, attacking the epithelial midgut cells of adult honey bees (Chen and Huang, 2010). It is first reported attacking *A. mellifera* in Taiwan, and has been found in Asia, Europe, and the Americas in the last decade. *N. ceranae* infection has been found to affect honey bee behaviour and physiology (Goblirsch *et al.*, 2013), to induce energy stress (Mayack and Naug, 2009) and to cause immune suppression (Karina *et al.*, 2009). It causes colony mortality and is speculated to be a main reason for colony losses (Higes *et al.*, 2007).

In this study, we intended to determine whether transportation and pollination service would affect the infection rate (prevalence) and the average number of spores per bee (abundance) of *N. ceranae* in workers of *A. mellifera*. We hypothesized that transportation and the ensuing pollination weakens the immune system of bees, rendering them more susceptible to *N. ceranae* infections, resulting in higher prevalence and abundance of *N. ceranae* in honey bee hosts.

Fourteen colonies were randomly divided into two groups. A group of "transported and pollinating colonies" (TP) was moved to pollination for blueberry for 11 days (17-28 May 2013). A group of

"stationary colonies" (S) was not moved, and served as the control during the same time. The S group stayed in East Lansing, MI, USA (GPS position: N42°40'44.97", W84°28' 39.16"), while the TP group was moved to Fennville, MI, USA (GPS position: N42°35'38.07", W86° 9'18.85"), with a distance between the two as 137.6 km. On 17 May, 15 foragers were sampled per colony from all 14 colonies using a bee vacuum, before transportation took place. These samples were named TP₀ and S₀. On 4 June, 15 foragers per colony were again sampled from the two groups (TP₁ and S₁). We waited seven days to do the 2nd sampling after the 2nd transportation (Fennville to East Lansing), to allow bees time to respond to the 2nd transportation trip.

We isolated the midgut of each bee, and homogenized it in 200 ml distilled water using a plastic pestle inside an Eppendorf tube. Spore number was determined using a hemocytometer chamber (Hausser Scientific) (Fries *et al.*, 2013). We ran PCR (Chen *et al.*, 2008) on 10 random samples and identified all 10 bees to be infected by a single species of *N. ceranae*.

We used Log (x+1) and arcsine transformations for spore load and infection rate, respectively to stabilize their variance and used one-tailed T-tests when comparing treatments with specific *a priori* predictions, and two-tailed T-tests for others. Analysis was done by Statview 5.0.1 (SAS Institute; Gary, NC, USA).

There was no significant difference in infection rates between TP₀ and S₀ (T = 0.013, 2-tailed test, P = 0.99) and between S₀ and S₁ (T = 0.43, 2-tailed test, P = 0.67) (Fig. 1A). This suggests that TP and S groups had the same *Nosema* prevalence at the beginning of the experiment. In addition, prevalence of S group did not change before and after transportation and pollination. However, we found a significantly higher *Nosema* prevalence in TP₁ than S₁ (T = 2.14, 1-tailed test, P = 0.027), suggesting that transportation and/or pollination caused a higher infection rate in workers.

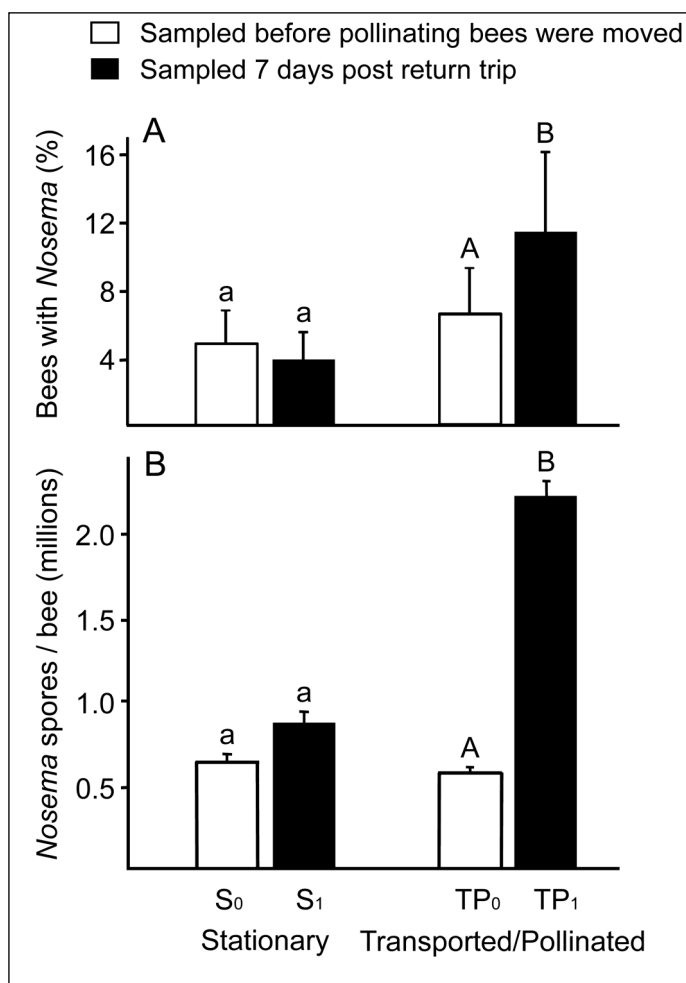


Fig. 1. Percent of bees (\pm SE) infected with *N. ceranae* (A) and mean number of *N. ceranae* spores (million, mean \pm SE) per bee (B) in four groups of bees. Bees either stayed in East Lansing, MI ("Stationary"), with no pollination commission, or were transported to Fennville, MI, for 11 days of blueberry pollination and transported back (total distance travelled 270 km, "TP"). Each group had seven colonies with 15 workers sampled per colony. Different letters on top of bars indicate significant difference at $P < 0.05$ with either one-tailed T-test (TP₁ vs. TP₀, TP₁ vs. S₁) or two-tailed T-tests (other comparisons). Comparisons were done only within each treatment before and after (0 and 1). Data presented were untransformed but statistics were performed post transformation.

Nosema abundance followed a similar pattern (Fig. 1B). There was no significant difference between the TP₀ and S₀ ($T = 0.44$, 2-tailed test, $P = 0.67$), and between S₀ and S₁ ($T = 0.37$, 2-tailed test, $P = 0.71$). Therefore TP and S had the same *Nosema* abundance before bees were moved for pollination service and S colonies did not change their *Nosema* abundance during the same period. However *Nosema* abundance was significantly higher in TP₁ than TP₀ ($T = 1.72$, 1-tailed test, $P = 0.05$). TP₁ was almost significantly higher than S₁ ($T = 1.63$, 1-tailed test, $P = 0.06$), and the abundance of TP₁ (2,223,295 spores per bee) was more than 2.5 times as many as S₁

(860,191 spores per bee).

In conclusion, the major finding in this study is that transportation and pollination lead to higher *Nosema* prevalence and abundance in worker bees. It is not clear which one is the main cause because transportation and pollination were confounded in this study so their effects could not be separated. However, it is more likely that it is mainly due to transportation (and its subsequent displacement for two times) because a recent study showed that bees placed in blueberry fields for pollination had almost no blueberry pollen (Pettis *et al.*, 2013), suggesting that bees did not have a monofloral diet when placed at blueberry fields (at least for pollen). It is also not clear whether these increases in pathogens were due to reduced immune system in the host bees. Future studies are needed to determine how stressful the pollination service alone is to honey bees.

Acknowledgements

We thank Meghan O Milbrath for help in the course of this project. Melissa S Huang, Meghan O Milbrath and Jianke Li helped improve this manuscript. This project was funded by Managed Pollinator CAP grant from Agriculture and Food Research Initiative Competitive Grant No. 20098511805718, from the USDA National Institute of Food and Agriculture and an earmarked fund from China Agriculture Research System (CARS-45-KXJ11).

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