Note

Black queen cell virus and drifting of honey bee workers (*Apis mellifera*)

Gina Retschnig [a,b,*], Liv A. Kellermann [a,b,c], Marion M. Mehmann [a,b], Orlando Yañez [a,b], Pius Winiger [a,b], Geoffrey R. Williams [a,b,d], Peter Neumann [a,b]

[a] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Schwarzenburgstrasse 161, 3003 Bern, Switzerland
[b] Agroscope, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland
[c] Department of Agronomy, Bern University of Applied Sciences HAFL, Länggasse 85, 3052 Zollikofen, Switzerland
[d] Department of Entomology & Plant Pathology, Auburn University, 301 Funchess Hall, Auburn, AL 36849, USA

*Correspondence: E-mail: gina.retschnig@vetsuisse.unibe.ch,
Tel.: +41 (0)31 631 57 68
ORCID ID: 0000-0001-6249-9853

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Summary

Social insects may accidentally drift into foreign nests due to orientation errors. Even though pathogens have been reported to promote drifting, no data currently exist about the potential impact of titers of the widespread black queen cell virus (BQCV) on the orientation abilities of honey bee workers, *Apis mellifera*. Here, we investigated titers of BQCV in naturally infected drifted and non-drifted workers. The data show significantly higher virus titers in the drifted workers (Wilcoxon rank sum test, $P < 0.01$). Our results suggest that high BQCV loads may compromise honey bee orientation, possibly by affecting learning performance similar to other viruses. If future work demonstrates that the correlation found here represents a causal relationship between higher viral titers and drifting, this will be the first identification of clinical symptoms of BQCV in adult honey bee hosts.

43 Honey bees may not return to their home colonies due to orientation errors, which
44 is known as drifting (Rauschmayer 1928; Neumann et al. 2000). Infections with
45 pathogens might enhance chances of orientation errors, thereby promoting both drifting
46 and pathogen transmission between colonies (Fries and Camazine 2001). While honey
47 bee viruses are common (Chen and Siede 2007), no significant association between the
48 presence of ten common viruses and drifting of honey bee workers has been reported
49 yet (Forfert et al. 2015). However, there may be an important difference with respect
50 to phenotype between the sheer detection of a pathogen vs. the actual pathogen load,
51 i.e. only higher virus loads may affect honey bee behavior as in case of learning and
52 deformed wing virus (Iqbal and Mueller 2006). Here, we compared for the first time
53 natural infection levels with the nearly ubiquitous black queen cell virus (BQCV)
54 between drifted and non-drifted honey bee workers.

55 For this study, freshly emerged honey bee workers from four local colonies were
56 individually marked and randomly introduced into three queenright 2-frame
57 observation hives (N=200 each) that were installed in one row at intervals of ~1.5 m;
58 each was equipped with an optically distinguishable hive entrance. All labelled workers
59 that were present in the hives after 14 days were recaptured (N=178) and assigned to
60 drifting status based on their markings (drifted N=13; non-drifted N=165). Then,
61 BQCV levels were quantified using standard qPCR (Gauthier et al. 2007). Briefly, total
62 RNA of individually homogenized workers was extracted using the Nucleospin RNA
63 II kit (Macherey-Nagel, Düren, Germany) and reverse transcription was conducted with
64 the ThermoscriptTM RT system (Invitrogen, Carlsbad, USA) according to
65 manufacturer guidelines. qPCR was then performed using a KAPA SYBR FAST
66 Universal Mastermix kit (KAPA Biosystems, Wilmington, USA) in an Eco™ Real

Time PCR System (Illumina, San Diego, USA) (de Miranda et al. 2013) with specific primers for BQCV published in Gauthier et al. 2007.

The analyses showed a significant effect of the observation hive on drifting ($\chi^2 = 10.52$, $P < 0.01$): 15.39 %, 4.62 % and 0 % of the bees drifted from hive 1, 2 and 3, respectively. Despite the limited sample size of drifting workers (N=13), they showed significantly higher BQCV titers compared to non-drifting ones (N = 165, Wilcoxon rank sum test, $P < 0.01$) (Figure 1).

In a previous study, no significant differences in the prevalence of BQCV and nine other common viruses were found between drifted and non-drifted honey bee workers (Forfert et al. 2015). However, the significantly higher viral titers of drifted workers in our study suggest that only high BQCV loads may compromise orientation abilities of their honey bee hosts, very similar to effects on learning performance by deformed wing virus (Iqbal and Mueller 2006). If that holds true, this compromised orientation ability would constitute the first reported clinical symptom of BQCV in adult honey bees (Chen and Siede 2007).

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Figure I

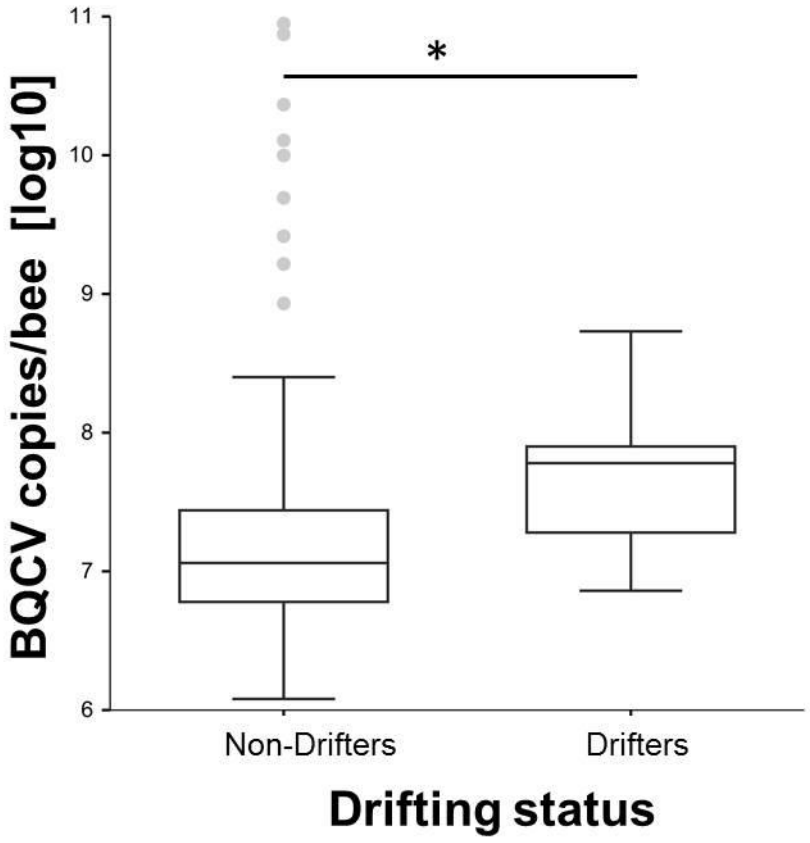

Figure I. Drifting of adult worker honey bees and BQCV copies/bee. Drifted workers (N = 13) showed significantly higher BQCV titers compared to non-drifted ones (N = 165; Wilcoxon rank sum test, $P < 0.01$=*). Boxplots show the inter-quartile range (box), median (black line within box), data range (vertical lines) and outliers (grey dots). NCSS 10 was used for statistical analyses and the Figure.

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