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Data in brief

Brain transcriptomes of honey bees (*Apis mellifera*) experimentally infected by two pathogens: Black queen cell virus and *Nosema ceranae*

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ABSTRACT

Regulation of gene expression in the brain plays an important role in behavioral plasticity and decision making in response to external stimuli. However, both can be severely affected by environmental factors, such as parasites and pathogens. In honey bees, the emergence and re-emergence of pathogens and potential for pathogen co-infection and interaction have been suggested as major components that significantly impaired social behavior and survival. To understand how the honey bee is affected and responds to interacting pathogens, we co-infected workers with two prevalent pathogens of different nature, the positive single strand RNA virus Black queen cell virus (BQCV), and the Microsporidia *Nosema ceranae*, and explored gene expression changes in brains upon single infections and co-infections. Our data provide an important resource for research on honey bee diseases, and more generally on insect host-pathogen and pathogen-pathogen interactions. Raw and processed data are publicly available in the NCBI/GEO database: (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE81664.

Specifications	
Organism/cell line/tissue	*Apis mellifera carnica* brains
Sex	Female workers, 15 days old
Sequencer or array type	Illumina HiSeq 2000
Data format	Raw and analyzed data
Experimental factors	Uninfected controls vs. BQCV infected vs. *N. ceranae* infected vs. BQCV + *N. ceranae* co-infected
Experimental features	Two days old worker bees were fed with the two pathogens, alone or in combination, or a control solution, and kept in cages for 13 days.
Consent	Non applicable
Sample source location	Three honey bee colonies from Institute for Biology of the Martin-Luther-University, in Halle (Saale), Germany. Inocula of naturally occurring pathogens were obtained from propagations in the laboratory.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81664

2. Introduction

The brain constitutes the central component of the insect nervous system. The regulation of gene expression in the brain plays an important role in behavioral plasticity and decision making in response to external stimuli [1]. Social insects such as honey bees represent good models to study the relationship between brain gene expression (i.e. neurogenomics) and behavioral modulations [2–4]. Insect societies are composed of reproductive females (queens), males and non-reproductive workers that each display a remarkably distinct behavioral repertoire. Workers in particular exhibit striking patterns of division of labor and behavioral maturation that are crucial for colony survival and growth, which generally consist of a sequence of behaviors known as temporal polyethism, from nursing and nest construction to nest guarding and food foraging [5]. Several external factors may modify this behavioral sequence, such as food availability and colony demography [5]. Pathogen infections also accelerate maturation towards early foraging, a change in behavior that is considered to function as a form

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of social immunity in insect societies [6]. Pathogens such as Microsporidian and viruses have been shown to accelerate temporal polyethism in honey bee colonies [7–9] as well as dramatically altering brain gene expression, including genes involved in neural function and foraging behavior [10].

Among the multiple pathogens infecting honey bees [11], the gut parasite *Nosema ceranae*, a Microsporidia which recently switched from its original host, the Eastern honey bee *Apis cerana*, to the Western honey bee *A. mellifera*, is one of the most prevalent parasite of honey bees in Europe [12]. Honey bees are also infected by several RNA viruses [13], and one of the most prevalent is the positive single strand RNA virus, Black queen cell virus (BQCV) [14].

In a previous study, we showed that BQCV and *N. ceranae* interact synergistically to increase worker bee mortality [15]. Using samples from the same experiment, we sequenced the brain transcriptome of worker bees infected by the two pathogens, alone or in combination, and compared it to control bees. Here, we explore the genome-wide response of worker bee brains to experimental infection.

3. Materials and methods

3.1. Experimental infections

Workers honey bees *Apis mellifera carnica* originated from colonies located in Halle (Saale), Germany. Colonies had been treated to control *Varroa* mites with Varidol® (Amitraz; TolnAgro, Hungary) the previous fall, six months before the experiment. Two day-old worker honey bees were experimentally infected individually with 10^5 *N. ceranae* spores and 1.4×10^9 genome equivalents of BQCV per os, alone or in combination (see details in [15]). Bees were kept 13 days post-infection in metal cages ($10 \times 10 \times 6$ cm) comprising 30 individuals from the same colonies and treatment, with an 8 cm^2 piece of organic beeswax. Cages were held in incubators at 30 °C ± 1 and 50% RH and bees were fed 50% sucrose solution ad libitum following standardized guidelines [16]. Three replicates using three different honey bee colonies were used within a treatment, and the same colonies were used across treatments.

3.2. RNA-sequencing

At the end of the experiment, bees were flash frozen in liquid nitrogen and brains dissected on dry ice. RNA was extracted from a pool of four brains (from all four treatments and three replicates except co-infection, for which only two replicates could be analyzed) using Trizol and the RNeasy Mini Kit (Qiagen). Sample preparation was performed using the DGE *Dpn*II Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA) with 2 μg RNA. Library preparation of mRNA was perform following the TruSeq Stranded mRNA Sample Preparation Kit from Illumina.

3.3. Data analysis

Image analysis and base calling were performed using the HiSeq Control Software and Real-Time Analysis component. Demultiplexing was performed using Illumina's sequencing analysis software CASAVA 1.8.2. The quality of the data was assessed using FastQC from the Babraham Institute and the Illumina software SAV (Sequence Analysis Viewer). Reads were mapped to the *Apis mellifera* genome (Amel_4.5) using the eland_rna module of CASAVA 1.8.2. NCBI annotation file (seq_gene.md.gz; 2012-12-17) was used to generate splice junctions automatically. Reads were also aligned to a set of contaminants, including the ribosomal RNAs, the PhiX genome (Illumina control) and the Illumina adapters. Reads mapping to contaminants were discarded. Gene counting was performed with HTSeq count (union mode). As the sequencing was strand-specific, the reads were mapped to the opposite strand of the gene. Before any statistical analysis, genes with <15 reads summed across all the analyzed samples were filtered out. Differentially expressed genes were identified using the Bioconductor R package *edgeR* 2.6.2 and the Upper Quartile normalization method [17]. Genes with adjusted *p*-value <5% (according to the Benjamini-Hochberg FDR method [18]) were declared differentially expressed. Functional analysis based on GO terms was performed using the online platform DAVID 6.7 [19]. Overlap tests with previously published brain transcriptomes upon parasitism with *Varroa* mites and *N. ceranae* [10] were performed using a hypergeometric test.

4. Results

A total of 875,319,378 reads were generated, with an average of 79,574,489 per replicate (±5,023,017 sem). After quality control and alignment to the *A. mellifera* genome, 397,450,508 reads (average per replicate: 36,131,864 ± 3,629,065 reads) were uniquely assigned to exons and used for statistical analysis of the host response to the experimental inoculation treatments (details in Supplementary Table 1).

The number of genes showing significant changes in expression level upon infection was markedly different between treatments. While 144 genes where differentially expressed in brains of workers infected by BQCV, only 13 genes had a different level of expression in brains of bees infected by *N. ceranae* (Fig. 1). Co-infection with the two pathogens induced the differential expression of 67 genes, including 29 genes that were also differentially expressed in brain of worker bees infected by BQCV, and 6 genes also differentially expressed in brain of bees infected by *N. ceranae*; 31 genes were differentially expressed in co-infected bees only. Among the latter, three cytochrome oxidase P450 genes (*LOC408453*, *CYP4G11* and *LOC412209*) and two genes coding for odorant binding proteins (*OBP4* and *OBP18*) were significantly down-regulated in co-infected bees only. Conversely, the gene coding for the protein *yellow-x1* (*LOC724293*) was significantly up-regulated in co-infected bees. Finally, one gene coding for a heat shock protein (*LOC724488*) was consistently down-regulated after all pathogen treatments, including co-infection.

The functional analysis of genes differentially regulated showed no significantly overrepresented GO terms in workers bees infected with *N. ceranae* or co-infected with both pathogens. However, we found a significant overrepresentation of genes involved in immune functions that were differentially expressed in brains of bees infected with BQCV (Benjamini-Hochberg corrected $p = 0.039$). Several genes from the Toll and Imd pathways were up-regulated, such as the antimicrobial peptides *abaecin* (*LOC406144*), *apidaecin* (*Apid1* and *Apid73*) and *hymenoptaecin* (*LOC406142*), but also *Rel*, *Lys-2*, the Drosophila homolog of *PIRK* (*LOC100578156*), and the gene coding for the pathogen recognition protein *PGRP-S2*. More importantly, two genes from the RNAi antiviral pathway, *AGO2* and *Dicer* (*LOC726766*), were found up-regulated in both treatments including BQCV. Although no functional group associated to brain and neuronal activities were overrepresented in the list of differentially expressed genes, we found the chemosensory protein *CSP6*, a light sensitive protein (*Lop2*), the heat shock cognate protein *Hsc70-4* and a neuropeptide CCHamide-1 receptor-like (*LOC411632*) to decrease in expression upon infection by BQCV. Infection by *N. ceranae* induced lower expression of the chemosensory protein *CSP1* but increased expression of the neurotransmitter *NT-4*. A complete list of genes exhibiting significant differential expression between treatments and control is available in Supplementary Table 2.

Comparison with a previous study investigating effect of the *Varroa* mite and *N. ceranae* on honey bee worker brain gene expression [10] revealed significant overlaps, with 27 and 2 genes found in response to *Varroa*/BQCV and both *N. ceranae* studies, respectively, in independent experiments (Supplementary Table 3).

5. Discussion

This study reports the transcriptome responses of the honey bee brain to two different pathogens, an RNA virus and a gut Microsporidia.

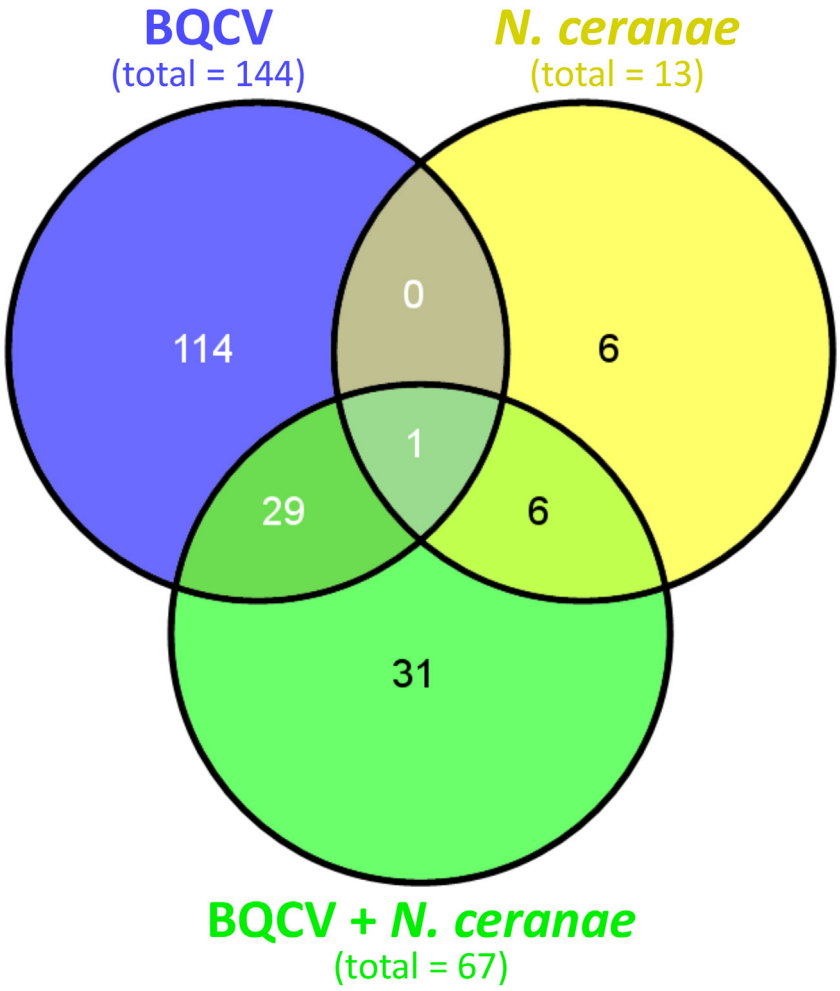

Fig. 1. Venn diagram showing the number of significantly differentially regulated gene transcripts between control and experimentally infected worker honey bees, and overlaps between the different experimental inoculation treatments with the two honey bee pathogens.

We found that BQCV induces a more dramatic change in gene expression than the Microsporidia *N. ceranae* in brains. The main reason is likely the capacity of the virus to reach and infect the central nervous system of its host, while *N. ceranae* is strictly restricted to the gut of honey bees. Such a difference of impact was also observed in a previous study comparing the effect of the *Varroa* mite when transmitting another RNA virus, Deformed wing virus (DWV), and the same Microsporidia *N. ceranae* [10]. Interestingly, we found that genes from the antibacterial/antifungal pathways Toll and Imd, including several AMPs, where not triggered upon infection with *N. ceranae*, but rather involved in the response to BQCV. Although observed in other model insect species [20], the involvement of these pathways in the antiviral response remains to be elucidated [21]. More importantly, we confirmed experimentally the role of the RNAi genes *Dicer* and *AGO2* in the antiviral response of honey bees, as previously observed in response to another virus [22].

An important aspect of our transcriptome study is that the response of the host to co-infection with two pathogens was also analyzed. BQCV and *N. ceranae* have been shown to interact synergistically in honey bee workers, with co-infection significantly decreasing host survival [15]. With these transcriptome sequences we identify candidate genes involved in pathogen interactions. For instance, genes involved in the behavior and responses to external stimuli such as *yellow-x1* [23] and odorant binding proteins [24] were found significantly differentially expressed in co-infected bees only. We believe that such data will provide important resource for research on honey bee diseases, and more generally on insect host-pathogen and pathogen-pathogen interactions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2016.09.010.

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