Analysis of genomic diversity and potential recombination in the microsporidian honey bee parasite *Nosema ceranae*

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**Objective**

To explore the possible contributions of PCR-mediated recombination to previous identified genomic diversity in *N. ceranae*.

Elucidating the origins of diversity in the *N. ceranae* genome will allow us to understand whether these parasites propagate clonally, or via sexually-related processes; a pre-requisite to help prevent future outbreaks of this disease.

**Background**

*Apis mellifera* (honey bee) colonies have experienced a multi-factorial global collapse over the past decade. The population decline has had grave effects on the economic sustainability of the beekeeping industry and agricultural practices that rely on insect pollination, notably monoculture.

The microsporidian parasite, *Nosema ceranae*, has been identified as a potential contributor to the collapse due to its increased prevalence in honey bee colonies and its detrimental effects on colony health.

Recent studies have observed high genetic variability within geographically distinct *N. ceranae* populations, some of which could be attributed to the presence of gene exchange and recombination. The presence of recombination is currently believed to occur as a result of meiotic-driven processes (i.e. sexual reproduction); a transient sexual phase where two genotypes of different lineages fuse and recombine to produce new variants.

Polymerase chain reaction (PCR)-mediated recombination has been documented to produce recombinant variants that mimic the presence of homologous recombination. It is possible that previous reports of recombination may represent artefactual results.

**Results**

Does DNA dilution have an effect on observed haplotypes?

More concentrated DNA samples resulted in an increased number of haplotypes.

Results indicate genetic diversity is affected by experimental conditions.

Does PCR-mediated recombination occur in vitro to produce new haplotypes?

More than 4 haplotypes observed after amplification of mixed sample; not all the original haplotypes were recovered.

Majority of haplotypes differ by only one nucleotide; possible result of simple polymerase error or template switching.

Novel haplotypes likely a result of PCR-mediated recombination.

**Methods**

To detect the presence of PCR-recombination, four haplotypes were isolated from a clone library, mixed together and amplified as a heterogeneous population.

As a control, the four haplotypes were amplified individually to verify purity of the clone library.

The presence of more than four haplotypes in the mixed sample post-amplification could be the product of PCR-mediated recombination.

**Summary**

PCR-mediated recombination has been proven to increase diversity in population studies. We show this phenomenon to be a possible culprit in the overestimation of genetic diversity in recent *N. ceranae* studies.

We conclude that *N. ceranae* most likely propagates clonally; the presence of the previously reported prominent sexual phase is weakly supported.

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