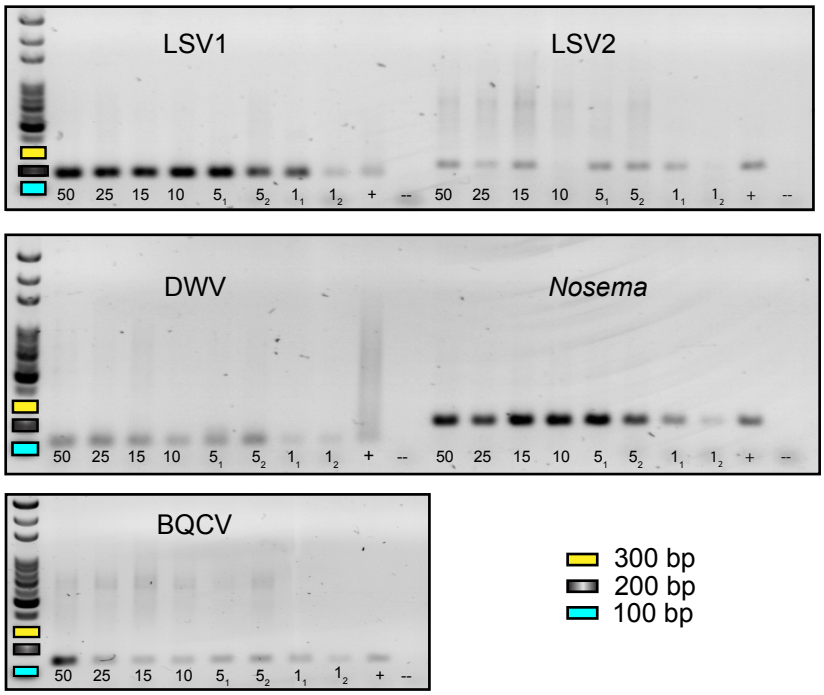
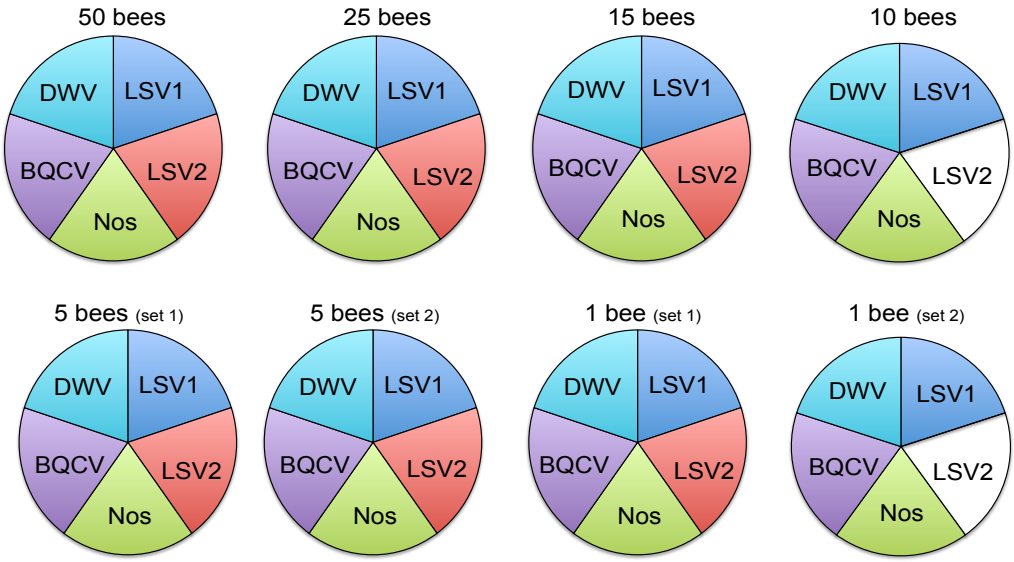
A



B



Supplemental Figure S10. Pathogen detection in RNA samples prepared from variable numbers of honey bees obtained from a single colony. Different quantities of honey bees (50, 25, 15, 10, 5, or 1) were homogenized (i.e., samples of ≥10 frozen bees were homogenized in sealed plastic bags using a marble rolling pin, then thoroughly mixed by shaking contents, prior to transferring 1 gram of material per sample to a microfuge tube (2 ml) for RNA extraction; samples of ≤5 bees were homogenized in microfuge tubes (2 ml) using 3mm glass beads (see Methods section for additional details)). Following RNA extraction and reverse transcription of a normalized amount of RNA (2000 ng) from each sample, pathogen-specific PCR was performed to identify the pathogens present in each sample. Samples from this representative colony tested positive for BQCV, DWV, LSV1, LSV2, and *Nosema ceranae*.

A. Images of LSV1, LSV2, DWV, *Nosema ceranae*, and BQCV PCR products after agarose gel electrophoresis; sample sizes (i.e., 50, 25, 15, 10, $5_{(set1)}$, $5_{(set2)}$, $1_{(set1)}$, and $1_{(set2)}$) are noted in each lane.

B. Graphical representation of pathogens detected in each sample. Colored regions of each pie-chart indicate that the pathogen was detected, whereas white areas indicate that the pathogen was not detected in that sample. The results from this representative experiment of 3 replicates (see Supplemental Table S6), indicate that a sample size of 5 adequately represents the majority of pathogens associated with a particular colony.