

## Multiyear survey targeting disease incidence in United States honey bees

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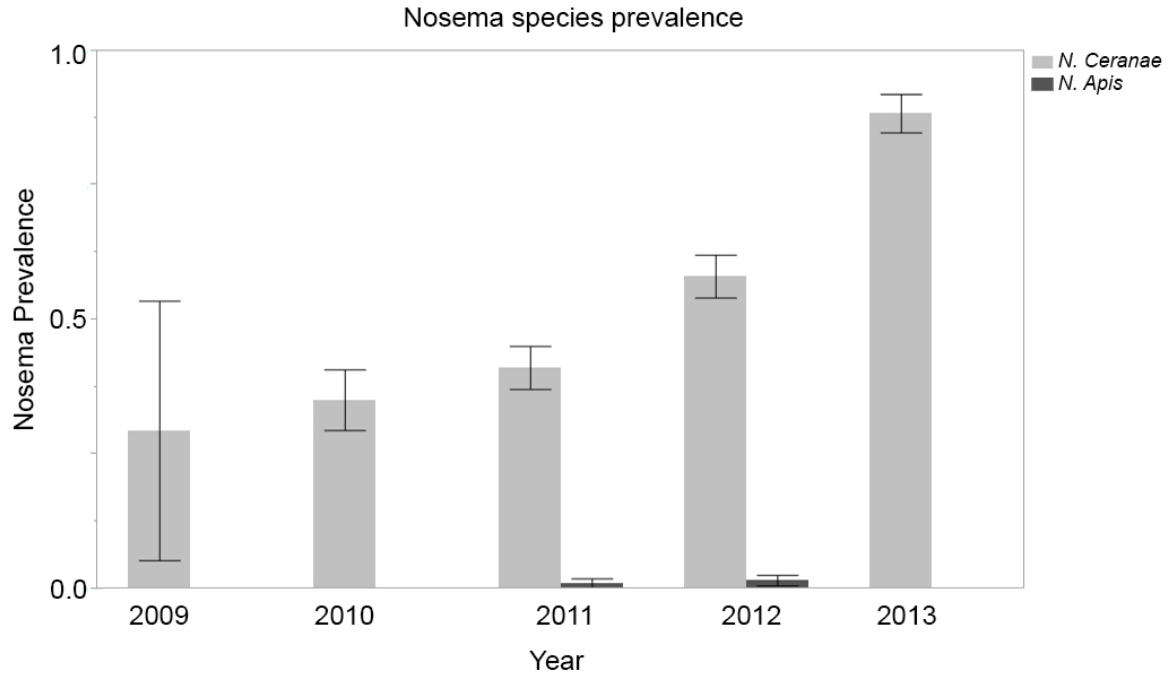
**Supplemental Table 1: RNA analysis assays and primers**

Survey	Year	Assay	Primer	Sequence	NT	Publication
a,b,c	2009-12	RpS5	RPS5.F	AATTATTTGGTCGCTGGAATTG	22	
			RPS5.R	TAACGTCCAGCAGAATGTGGTA	22	vanEngelsdorp et al., 2009
a,b,c	2009-12	DWV	DWV.F	GAGATTGAAGCGCATGAACA	20	
			DWV.R	TGAATTCAGTGTGCCCCATA	20	vanEngelsdorp et al., 2009
a,b,c	2009-12	BQCV	BQCV.F	TTTAGAGCGAATTCGGAAACA	21	
			BQCV.R	GCCGTACCGATAAAGATGGA	20	vanEngelsdorp et al., 2009
a,b,c	2009-12	IAPV	IAPV-F1a F	GCGGAGAATATAAGGCTCAG	20	
			IAPV-F1a R	CTTGCAAGATAAGAAAGGGGG	21	vanEngelsdorp et al., 2009
a,b,c	2009-12	IAPV	IAPV-F6627	CCATGCCTGGCGATTAC	18	
			KIABPV-B6707	CTGAATAATACTGTGCGTATC	21	Locke et al, 2012
a,b,c	2009-12	ABPV	ABPV.F	ACCGACAAAGGGTATGATGC	20	
			ABPV.R	CTTGAGTTTGCGGTGTTCCT	20	vanEngelsdorp et al., 2009
a,b,c	2009-12	CBPV	CBPV.F	CAAATCAACGAGCCAATCA	20	
			CBPV.R	AGTGTGAGGATCACCGGAAC	20	This study
a,b,c	2009-12	KBV	KBV.F	TGAACGTGACCTATTGAAAAA	22	
			KBV.R	TCGATTTTCCATCAAATGAGC	21	vanEngelsdorp et al., 2009
a,b,c	2009-12	SBPV	SBPV.F	CGCAAACACGACGAATTTTA	20	
			SBPV.R	GGTGCATAAACAGAATGACGAG	23	This study
a,b,c	2009-12	<i>Nosema ceranae</i>	Mncer143.F	CGTTAAAGTGTAGATAAGATGTT	23	
			MnUni.R	GACTTAGTAGCCGTCTCTC	19	Fries et al., the BEE BOOK
a,b,c	2009-12	<i>Nosema apis</i>	NAU.F	CTGCGAGGATATGATCTGAGGATG	24	
			NAU.R	CACTAATAGTGTGAGTCTAATAAAGGGTCTC	31	Scwarz, Evans 2013
d	2012-13	RpS5	RPS5.F	AATTATTTGGTCGCTGGAATTG	22	
			RPS5.R	TAACGTCCAGCAGAATGTGGTA	22	vanEngelsdorp et al., 2009
d	2012-13	DWV	DWV.F	GAGATTGAAGCGCATGAACA	20	
			DWV.R	TGAATTCAGTGTGCCCCATA	20	vanEngelsdorp et al., 2009

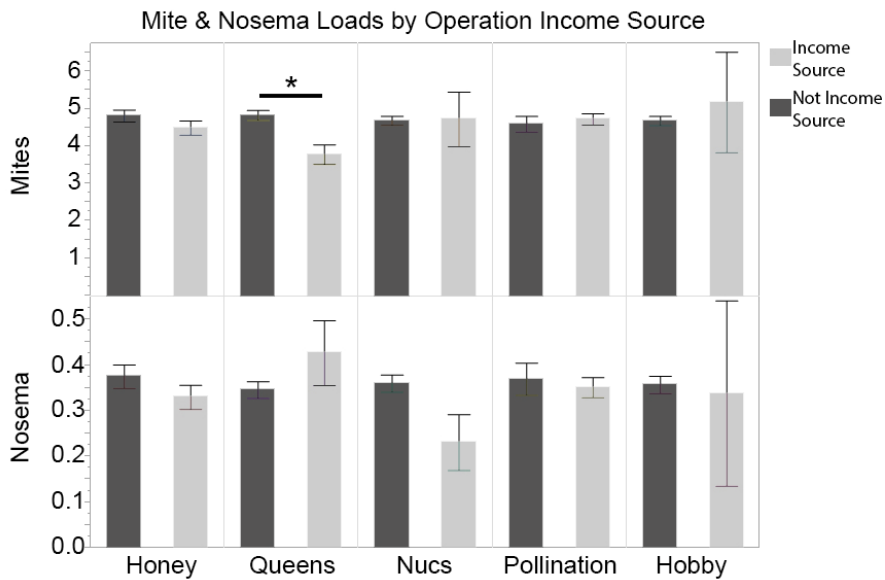
d	2012-13	BQCV	BQCV.F	TTTAGAGCGAATTCGGAAACA	21	
			BQCV.R	GCGGTACCGATAAAGATGGA	20	vanEngelsdorp et al., 2009
d	2012-13	IAPV	IAPV-F1a F	GCGGAGAATATAAGGCTCAG	20	
			IAPV-F1a R	CTTGCAAGATAAGAAAGGGGG	21	vanEngelsdorp et al., 2009
d	2012-13	IAPV	IAPV-F6627	CCATGCCTGGCGATTAC	18	
			KIABPV-B6707	CTGAATAATACTGTGCGTATC	21	Locke et al, 2012
d	2012-13	ABPV	ABPV.F	ACCGACAAAGGGTATGATGC	20	
			ABPV.R	CTTGAGTTTGCGGTGTTTCT	20	vanEngelsdorp et al., 2009
d	2012-13	CBPV	CBPV.F	CAAATCAACGAGCCAATCA	20	
			CBPV.R	AGTGTGAGGATCACCGAAC	20	This study
d	2012-13	KBV	KBV.F	TGAACGTCGACCTATTGAAAAA	22	
			KBV.R	TCGATTTTCCATCAAATGAGC	21	vanEngelsdorp et al., 2009
d	2012-13	SBPV	SPV-F3177	GCGCTTTAGTTCAATTGCC	19	
			SPV-B3363	ATTATAGGACGTGAAAATATAC	22	Locke et al, 2012
d	2012-13	<i>Nosema ceranae</i>	Mncer143.F	CGTTAAAGTGTAGATAAGATGTT	23	
			MnUni.R	GACTTAGTAGCCGTCTCTC	19	Fries et a., the BEE BOOK
d	2012-13	<i>Nosema apis</i>	NAU.F	CTGCGAGGATATGATCTGAGGATG	24	
			NAU.R	CACTAATAGTGTGAGTCTAATAAAGGGTCTC	31	Scwarz, Evans 2013
e	2013-14	DWV	DWV-F8668	TTCATTAAGCCACCTGGAACATC	24	
			DWV-B8757	TTTCCTCATTAACTGTGTCGTTGA	24	Locke et al, 2012
e	2013-14	ABPV	ABPV-F6548	TCATACCTGCCGATCAAG	18	
			KIABPV-B6707	CTGAATAATACTGTGCGTATC	21	Locke et al, 2012
e	2013-14	IAPV	IAPV-F6627	CCATGCCTGGCGATTAC	18	
			KIABPV-B6707	CTGAATAATACTGTGCGTATC	21	Locke et al, 2012
e	2013-14	SBPV	SPV-F3177	GCGCTTTAGTTCAATTGCC	19	
			SPV-B3363	ATTATAGGACGTGAAAATATAC	22	Locke et al, 2012
e	2013-14	RP49 mRNA	RP49-qF	AAGTTCATTGTCACCAGAG	20	
			RP49-qB	CTTCCAGTTCCTTGACATTATG	22	Locke et al, 2012
e	2013-14	KBV	KBV.F	TGAACGTCGACCTATTGAAAAA	22	

			KBV.R	TCGATTTTCCATCAAATGAGC	20	vanEngelsdorp et al., 2009
e	2013-14	LSV-2	qLSV2-F1722	CGTGCTGAGGCCACGGTTGT	19	
			qLSV2-R1947	GCGGTGTCGATCTCGCGGAC	20	Runckel et al., 2011
e	2013-14	CBPV	CBPV1-qF1818	CAACCTGCCTCAACACAG	18	
			CBPV1-qB2077	AATCTGGCAAGGTTGACTGG	20	Locke et al, 2012

Supplemental Figures



**Fig. S1.** Mean prevalence for *N. ceranae* (light grey) and *N. apis* (dark grey) with 95% CI error bars plotted by year. *N. apis* was only detected in 2011 and 2012.



**Fig. S2.** Varroa mites per 100 bees (top) and *Nosema* spore counts (bottom), segregated by primary income source for operations that only indicated one income source. Error bars depict 95% CI. While pollination (N = 1095) and honey (N = 546) operations are widely represented, queen (N = 152) and nuc (N = 31) production are less widely surveyed, thus limiting analysis by month. Significant differences indicated,  $p < 0.05$  (\*)

## Supplemental Text

### S1: Exotic Pests & Viruses

One of the drivers for the NHBDS is surveillance for the introduction of *Tropilaelaps* spp.. This exotic honey bee mite successfully migrated from its original host *Apis dorsata*, the giant honey bee, to the European honey bee *A. mellifera* in Asia (Delfinado and Peng 1995; Rath, Boecking et al. 1995). Large-scale shipping routes between Asia and North America increase the likelihood of an inadvertent introduction. *Tropilaelaps* infestations can increase up to 25x more rapidly than *V. destructor* and both mites transmit viruses (Forsgren, de Miranda et al. 2009), thus *Tropilaelaps* could match or even exceed the impact of *Varroa*. To validate the absence of this devastating pest, the NHBDS focused on high-risk states (California (CA), Florida (FL) and Hawaii (HI)) and widespread monitoring for *Tropilaelaps* using the efficient “bump” technique (Pettis, Rose et al. 2013).

APHIS suspended imports of honey bee queens and package bees from Australia on December 21, 2010 (Animal and Plant Health Inspection Service 2010), due to importation risks of slow bee paralysis virus (SBPV) and the Asian honey bee *A. cerana*, discovered near Cairns, Queensland in Australia in May 2007 (Ryan 2010; Somerville 2011). *A. cerana* is a smaller, less desirable managed pollinator (Ryan 2010). In tropical areas (e.g. the Solomon Islands), *A. cerana* has been shown to out-compete *A. mellifera* in nectar and pollen gathering and exhibits a propensity for robbing European honey bee stores (Anderson 2012). The likely movement of disease agents from *A. cerana* to *A. mellifera* is a concern, due to shared pests such as *Varroa*.

SBPV is a rare honey bee virus that is difficult to survey because it kills its host rapidly. SBPV has been reported from Canada, the United Kingdom, Fiji, New Zealand, Western Samoa (Ellis and Munn 2005) and Australia (DAFF 2002; DAFF 2012). Like most honey bee viruses, SBPV persists naturally as a covert infection without symptoms, though the virus has also been shown to be transmitted by *V.*

*destructor* with lethal consequences (Carreck, Bell et al. 2010). Rarely found in honey bee colonies, it is possible that another organism is the primary host of this virus (Bailey & Ball, 1991; J. R. de Miranda et al., 2010). Due to the risks posed by SBPV should it arrive in the U.S., and to confirm the absence of this virus currently, a molecular assay was included as part of the NHBDS.

## **S2: Viruses surveyed**

We examined a suite of honey bee viruses for the National Honey Bee Disease Survey, including the still absent Slow bee paralysis virus (SBPV), as described above. When highly parasitized by *Varroa*, deformed wing virus (DWV)-infected bees show characteristic symptoms like crippled wings and foreshortened abdomens. The virus can be detected at high levels also in asymptomatic adult bees and all other life stages. *V. destructor* is an active, biological vector for the virus, and both the prevalence and the quantity of DWV in colonies tends to parallel the mite population. High levels of DWV can lead to the production of non-functional and short-lived worker bees, often resulting in colony death (de Miranda & Genersch, 2010).

Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV) are three closely related viruses. ABPV is the dominant species in Europe, KBV in the Americas, and IAPV in Australia (de Miranda, Bailey et al. 2013). They generally do not cause overt symptoms, but when transmitted by *V. destructor*, can be lethal (Todd, de Miranda, & Ball, 2007). All have been linked to CCD and similar phenomena in Europe and the U.S., although inconsistently and without causal proof (Cox-Foster et al., 2007; VanEngelsdorp et al., 2009; Berenyi, Bakonyi, Derakhshifar, Kogelberger, & Nowotny, 2006).

Chronic bee paralysis virus (CBPV) causes two distinct sets of symptoms i) trembling clusters of bees crawling at the hive entrance, and ii) hairless, shiny black bees often attacked by healthy bees (Ribière et al., 2008). The virus is transmitted orally and through direct contact, and therefore often



associated with overcrowding. CBPV is not known to induce massive colony losses, and the occurrence of this virus is sporadic, yet this species is a risk factor that can lead to weakened colonies (Ribiere, Olivier et al. 2010).

Black queen cell virus (BQCV), named for the blackened cell walls it causes in sealed queen cells and prepupae, is a very common virus throughout the world and is found in both adults and pupae. Despite the striking symptoms, this disease has not been linked to colony losses (Bailey & Ball, 1991). Although BQCV can be detected in *V. destructor*, its seasonal incidence and distribution is largely independent of mite infestation.

The Lake Sinai viruses (LSV-1, LSV-2) were only recently described (Runckel et al., 2011). LSV-1 is present throughout the year with a peak in early summer. LSV-2 has a very sharp incidence and abundance peak in late winter with low incidence and abundance the rest of the year. LSV-2 was found to be the most abundant single component in the honeybee microbiome in a recent study of a large scale migratory beekeeping operation in the US (Runckel et al., 2011). Our samples were screened for LSV-2, starting with survey effort E in 2013.