BeeDoctor and BeeClinic, two new diagnostic tools for bee health

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OUTLINE

- BEEDOC-project
- extension grade diagnostic tool: BeeDoctor
- limitations of molecular fingerprinting
- research grade diagnostic tool: BeeClinic
- working with BeeClinic
- conclusions

BEEDOC PROJECT

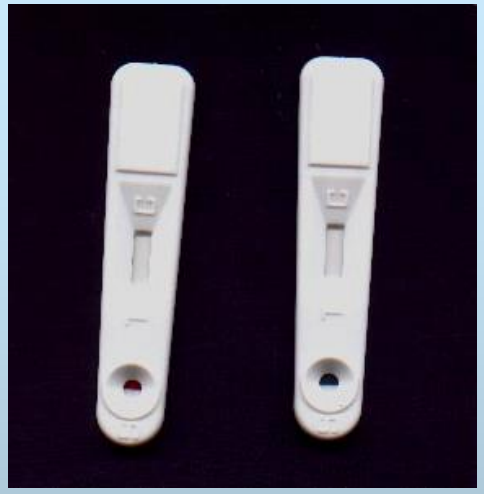
EU funded project < 7th framework programme
11 partners
diagnostics department

BEEDOC PROJECT

objectives:

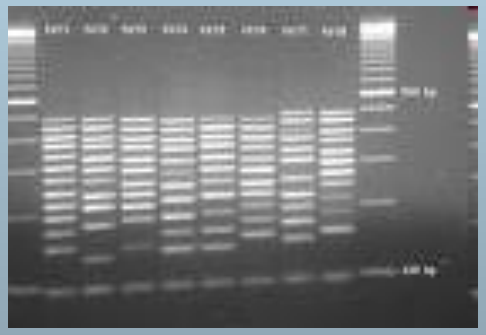
- field grade tool

= lateral flow device



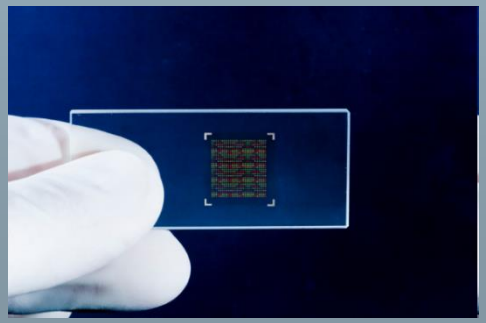
- extension grade tool

= BeeDoctor, PCR-based



- research grade tool

= BeeClinic, DNA chip



BEEDOC PROJECT

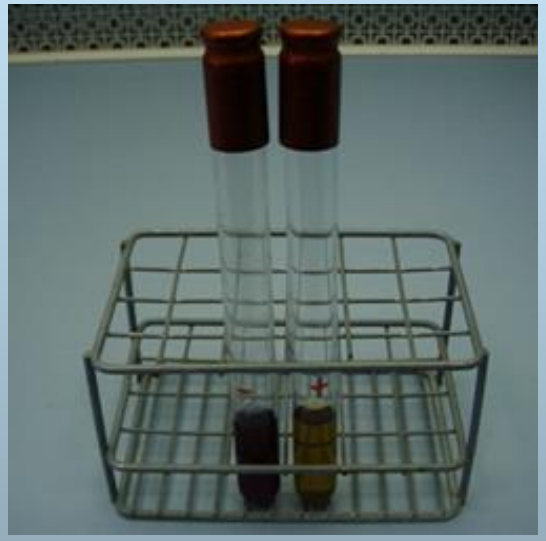
introduction of molecular diagnostics in bee health: early 2000

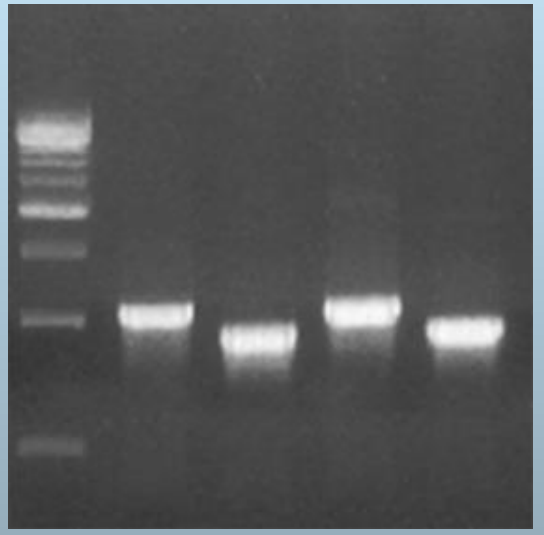
microscopy

CSI-like technology

BEEDOC PROJECT

introduction of molecular diagnostics in bee health:
American foulbrood





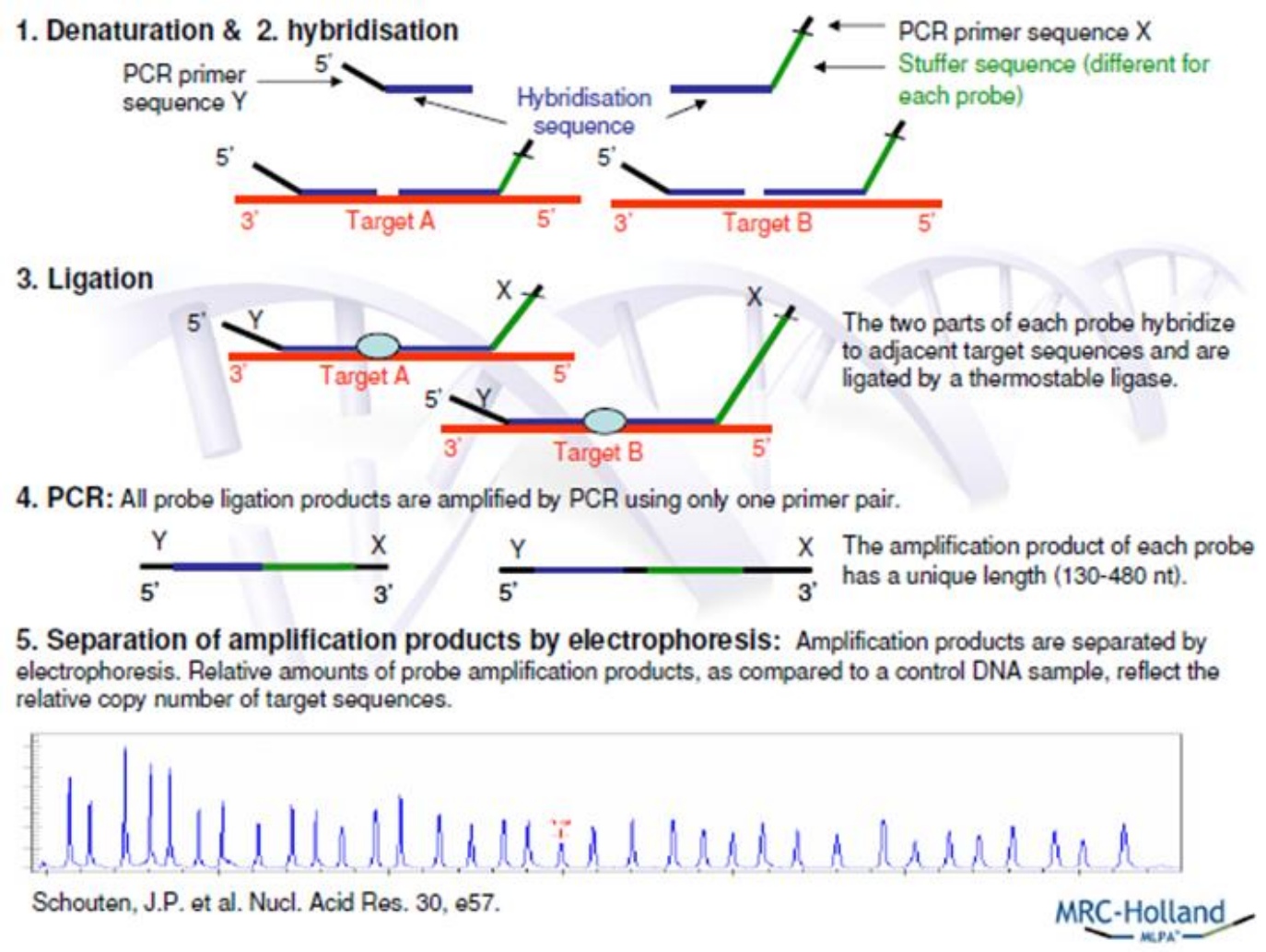
biochemical profiling
- cheap

PCR-technology
(molecular fingerprint)
- rapid
- highly specific

BEEDOCTOR

BeeDoctor

Multiplex Ligation-dependent Probe Amplification



BEEDOCTOR

example:

RespiFinder, 15 respiratory viruses in one reaction

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2008, p. 1232–1240 Vol. 46, No. 4
0095-1137/08/$08.00+0 doi:10.1128/JCM.02294-07

RespiFinder: a New Multiparameter Test To Differentially Identify Fifteen Respiratory Viruses▽

Martin Reijans,[1]* Gijs Dingemans,[1]† Corné H. Klaassen,[1] Jacques F. Meis,[1] Judith Keijdener,[2] Brit Mulders,[2] Kimberly Eadie,[3] Willem van Leeuwen,[3] Alex van Belkum,[3] Alphons M. Horrevorts,[1] and Guus Simons[2]

Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands[1]; PathoFinder BV, Maastricht, The Netherlands[2]; and Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands[3]

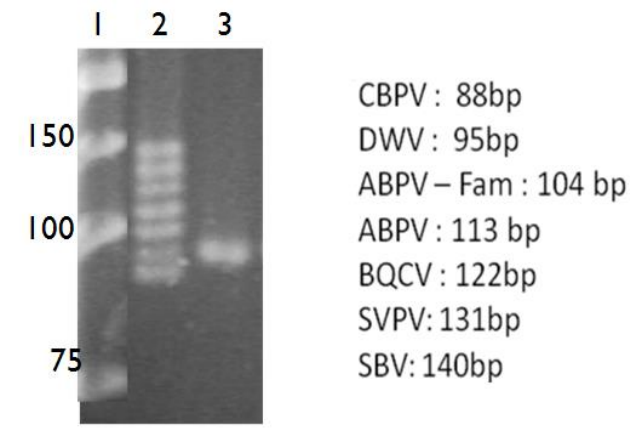
Received 29 November 2007/Returned for modification 2 January 2008/Accepted 25 January 2008

Broad-spectrum analysis for pathogens in patients with respiratory tract infections is becoming more relevant as the number of potential infectious agents is still increasing. Here we describe the new multiparameter RespiFinder assay, which is based on the multiplex ligation-dependent probe amplification (MLPA) technology. This assay detects 15 respiratory viruses in one reaction. The MLPA reaction is preceded by a preamplification step which ensures the detection of both RNA and DNA viruses with the same specificity and

BEEDOCTOR

results:

- targets = bee viruses:

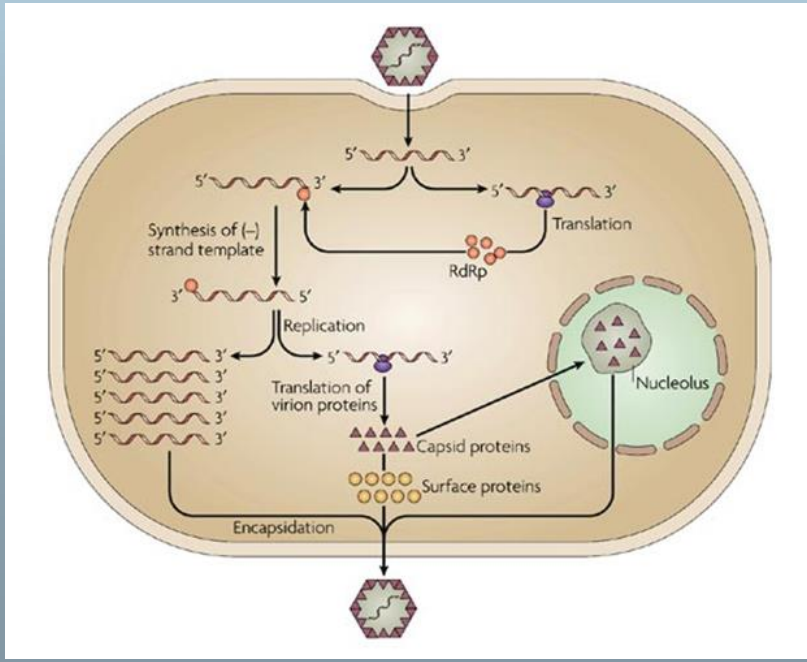


CBPV
DWV – KV – VDV
ABPV – KBV – IAPV
ABPV
BQCV
SBPV
SBV

- negative strand detection
- robust test

(single mutation permitted)

- proficiency test



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PLOS ONE

BeeDoctor, a Versatile MLPA-Based Diagnostic Tool for Screening Bee Viruses

Lina De Smet[1]*, Jorgen Ravoet[1], Joachim R. de Miranda[2], Tom Wenseleers[3], Matthias Y. Mueller[4], Robin F. A. Moritz[4], Dirk C. de Graaf[1]

1 Laboratory of Zoophysiology, Department of Physiology, Ghent University, Ghent, Belgium, **2** Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden, **3** Laboratory of Socioecology and Social Evolution, KU Leuven, Leuven, Belgium, **4** Institüt für Biologie, Martin Luther Universität Halle-Wittenberg, Halle/Saale, Germany

Abstract

The long-term decline of managed honeybee hives in the world has drawn significant attention to the scientific community and bee-keeping industry. A high pathogen load is believed to play a crucial role in this phenomenon, with the bee viruses being key players. Most of the currently characterized honeybee viruses (around twenty) are positive stranded RNA viruses. Techniques based on RNA signatures are widely used to determine the viral load in honeybee colonies. High throughput screening for viral loads necessitates the development of a multiplex polymerase chain reaction approach in which different viruses can be targeted simultaneously. A new multiparameter assay, called "BeeDoctor", was developed based on multiplex-ligation probe dependent amplification (MLPA) technology. This assay detects 10 honeybee viruses in one reaction. "BeeDoctor" is also able to screen selectively for either the positive strand of the targeted RNA bee viruses or the negative strand, which is indicative for active viral replication. Due to its sensitivity and specificity, the MLPA assay is a useful tool for rapid diagnosis, pathogen characterization, and epidemiology of viruses in honeybee populations. "BeeDoctor" was used for screening 363 samples from apiaries located throughout Flanders; the northern half of Belgium. Using the "BeeDoctor", virus infections were detected in almost eighty percent of the colonies, with deformed wing virus by far the most frequently detected virus and multiple virus infections were found in 26 percent of the colonies.

Citation: De Smet L, Ravoet J, de Miranda JR, Wenseleers T, Mueller MY, et al. (2012) BeeDoctor, a Versatile MLPA-Based Diagnostic Tool for Screening Bee Viruses. PLoS ONE 7(10): e47953. doi:10.1371/journal.pone.0047953

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Competing Interests: The authors have declared that no competing interests exist.

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BEEDOCTOR

first application: Flemish bee health monitoring

- virusscreening in 2011:

  363 samples from 170 apiaries

  30 adult bees taken at flight entrance

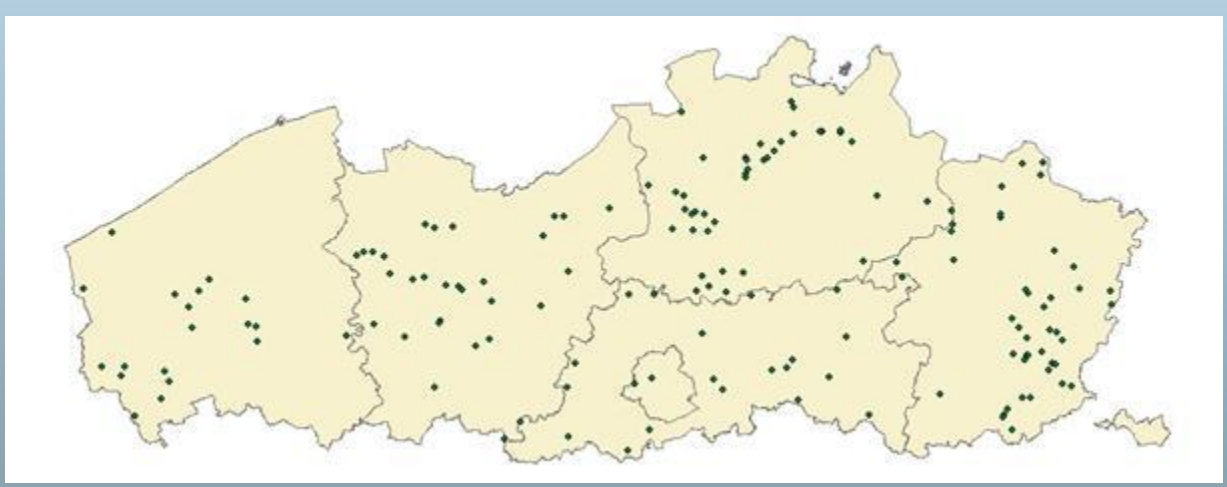

Table 2. Prevalence, co-infection rates and the results of the association analysis of honeybee viruses in Flemish apiaries.

			FREQUENCY	TOTAL PREVALENCE	ASSOCIATION ANALYSIS
ZERO VIRUSES	**TOTAL**	**78**	**21,5%**		n.a.
ONE VIRUS	ABPV	1	0,3%	3,3%	n.a.
	BQCV	14	3,9%	13,5%	n.a.
	CBPV	2	0,6%	1,7%	n.a.
	DWV	164	45,2%	69,4%	n.a.
	SBV	10	2,8%	19,0%	n.a.
	SBPV	0	0,0%	0,0%	n.a.
	TOTAL	**191**	**52,6%**		
					$\chi^2_{(1)}$
TWO VIRUSES	ABPV-BQCV	0	0,0%	-	$0,06^{n.s.}$
	ABPV-CBPV	0	0,0%	-	$0,06^{n.s.}$
	ABPV-DWV	9	2,5%	-	$0,04^{n.s.}$
	ABPV-SBV	1	0,3%	-	$0,03^{n.s.}$
	BQCV-CBPV	0	0,0%	-	$0,02^{n.s.}$
	BQCV-DWV	23	6,3%	-	$1,79^{n.s.}$
	BQCV-SBV	5	1,4%	-	$1,11^{n.s.}$
	CBPV-DWV	2	0,6%	-	$0,00^{n.s.}$
	CBPV-SBV	0	0,0%	-	$0,00^{n.s.}$
	DWV-SBV	45	12,4%	-	$2,19^{n.s.}$
	TOTAL	**85**	**23,4%**		
					$\chi^2_{(3)}$
THREE VIRUSES	ABPV-BQCV-CBPV	0	0,0%	-	$0,53^{n.s.}$
	ABPV-BQCV-DWV	0	0,0%	-	$4,47^{n.s.}$
	ABPV-BQCV-SBV	0	0,0%	-	$1,10^{n.s.}$
	ABPV-CBPV-DWV	1	0,3%	-	$0,42^{n.s.}$
	ABPV-CBPV-SBV	0	0,0%	-	$0,24^{n.s.}$
	ABPV-DWV-SBV	0	0,0%	-	$6,91^{P<0.10}$
	BQCV-CBPV-DWV	0	0,0%	-	$1,86^{n.s.}$
	BQCV-CBPV-SBV	0	0,0%	-	$1,12^{n.s.}$
	BQCV-DWV-SBV	7	1,9%	-	$5,33^{n.s.}$
	CBPV-DWV-SBV	1	0,3%	-	$1,94^{n.s.}$
	TOTAL	**9**	**2,5%**		
					$\chi^2_{(9)}$
FOUR VIRUSES	ABPV-BQCV-CBPV-DWV	0	0,0%	-	$3,09^{n.s.}$
	ABPV-BQCV-CBPV-SBV	0	0,0%	-	$2,22^{n.s.}$
	ABPV-BQCV-DWV-SBV	0	0,0%	-	$9,04^{n.s.}$
	ABPV-CBPV-DWV-SBV	0	0,0%	-	$5,94^{n.s.}$
	BQCV-CBPV-DWV-SBV	0	0,0%	-	$4,81^{n.s.}$
	TOTAL	**0**	**0,0%**		
					$\chi^2_{(21)}$
FIVE VIRUSES	ABPV-BQCV-CBPV-DWV-SBV	0	0,0%	-	$7,32^{n.s.}$
	TOTAL	**363**	**100,0%**		

doi:10.1371/journal.pone.0047953.t002

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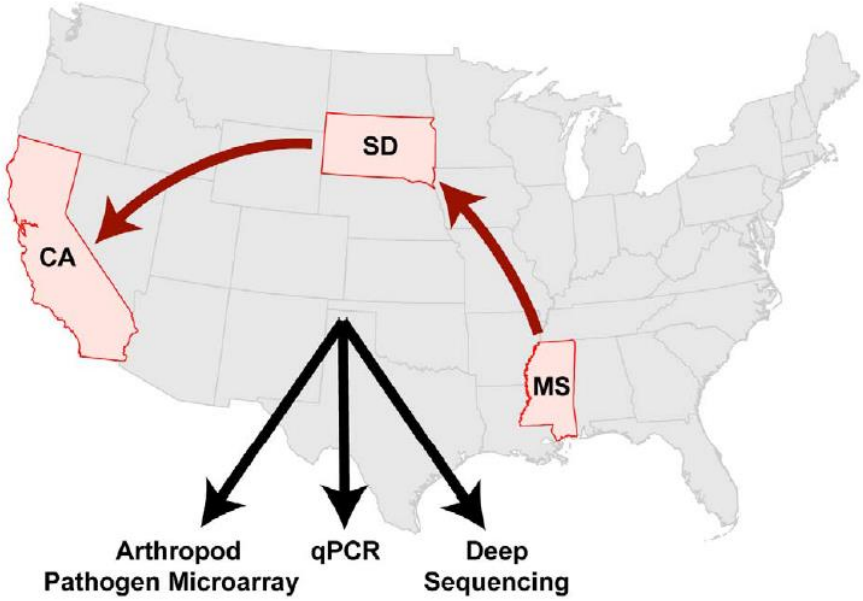
PLoS one

Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, *Nosema*, and *Crithidia*

Charles Runckel[1,2☯], Michelle L. Flenniken[3☯], Juan C. Engel[4], J. Graham Ruby[1,2], Donald Ganem[1,2], Raul Andino[3], Joseph L. DeRisi[1,2*]



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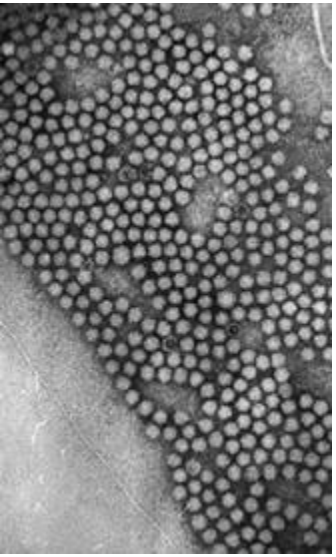
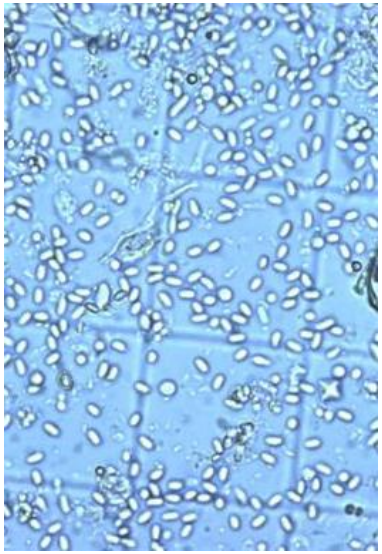
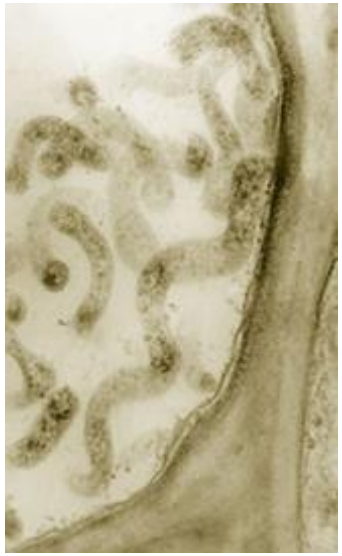


4 new viruses:
- ALPV str. Brookings
- BSRV
- LSV str. 1 and 2



Nosema ceranae

Crithidia mellificae



Spiroplasma melliferum and *S. apis*

Apocephalus borealis

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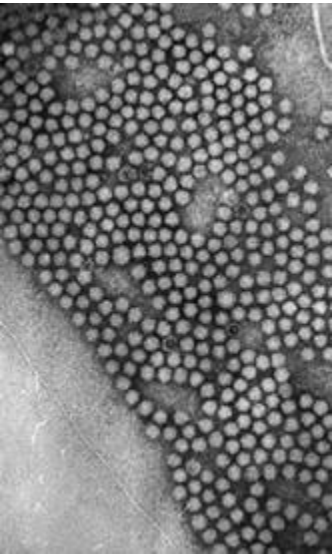
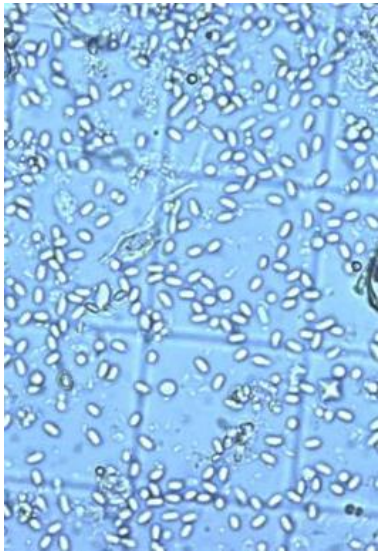
PLOS ONE

Comprehensive Bee Pathogen Screening in Belgium Reveals *Crithidia mellificae* as a New Contributory Factor to Winter Mortality

Jorgen Ravoet[1]*, Jafar Maharramov[2], Ivan Meeus[2], Lina De Smet[1], Tom Wenseleers[3], Guy Smagghe[2], Dirk C. de Graaf[1]

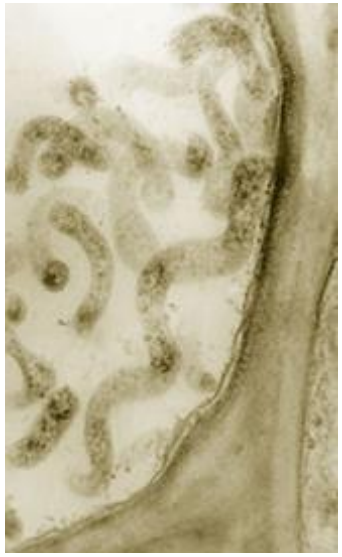


3 new viruses:
- ALPV str. Brookings
- VdMLV
- LSV str. 4



Nosema ceranae

Crithidia mellificae



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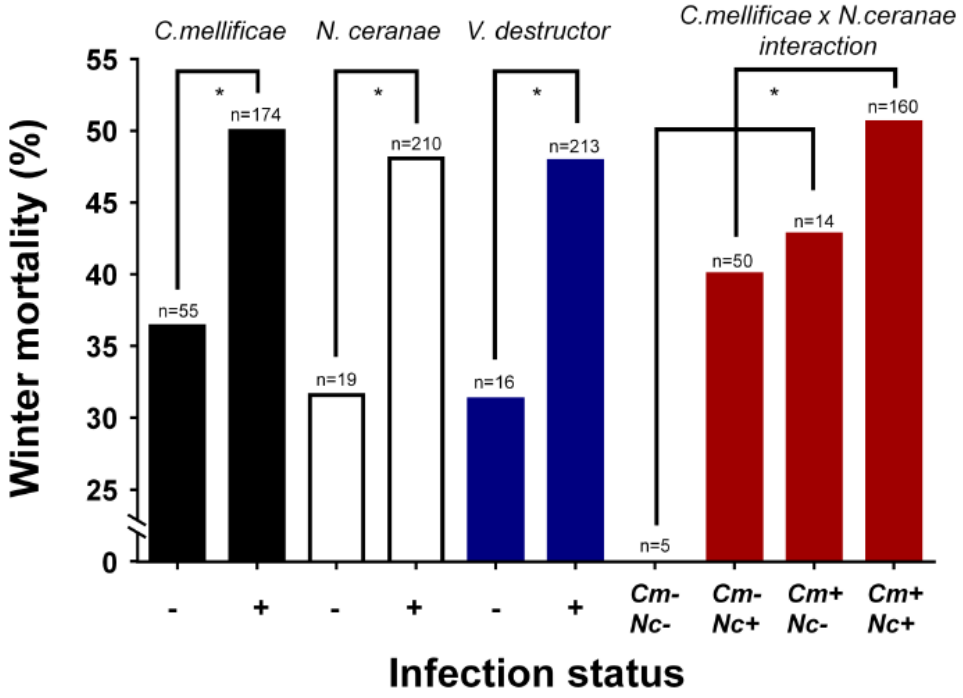

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Table 1. Honey bee pathogen incidences.

Pathogen	Type	Prevalences			Associations	
		Overall	Surviving colonies*	Collapsed colonies*	Between pathogens	With winter losses*
ABPV	Dicistroviridae	3.3% (12/363)	3.3% (4/122)	3.7% (4/107)		No
ALPV	Dicistroviridae	56.2% (204/363)	59.0% (72/122)	54.2% (58/107)	*Nosema* spores ($p = 0.011$)	No
Apicystis bombi	Ophryocystidae	40.8% (148/363)	41.8% (51/122)	41.1% (44/107)		No
Apocephalus borealis	Phoridae	31.1% (118/363)	32.8% (40/122)	33.6% (36/107)		No
BQCV	Dicistroviridae	13.5% (49/363)	10.7% (13/122)	14.0% (15/107)	LSV complex ($p = 0.009$)	No
CBPV	Unclassified RNA virus	1.7% (6/363)	0.0% (0/122)	1.9% (2/107)		No
Crithidia mellificae	Trypanosomatidae	70.5% (256/363)	71.3% (87/122)	81.3% (87/107)		Yes ($p = 0.03$)
DWV	Iflaviridae	69.4% (252/363)	61.5% (75/122)	67.3% (72/107)		No
LSV complex	Unclassified RNA virus	14.6% (43/363)	17.2% (21/122)	15.0% (16/107)	BQCV ($p = 0.009$)	No
Nosema apis	Nosematidae	10.2% (37/363)	13.1% (16/122)	10.3% (11/107)		No
Nosema ceranae	Nosematidae	92.6% (336/363)	89.3% (109/122)	94.4% (101/107)	VdMLV ($p < 0.001$)	No
Nosema spores	Nosematidae	75.2% (273/363)	71.3% (87/122)	72.9% (78/107)	ALPV ($p = 0.011$)	No
SBV	Iflaviridae	19.0% (69/363)	17.2% (21/122)	21.5% (23/107)		No
Spiroplasma apis	Spiroplasmataceae	0.3% (1/363)	0.0% (0/122)	0.0% (0/107)		No
Spiroplasma melliferum	Spiroplasmataceae	4.4% (16/363)	3.3% (4/122)	6.5% (7/107)		No
Varroa destructor	Varroidae	93.7% (313/334)	91.0% (111/122)	95.3% (102/107)		Yes ($p = 0.07$)
VdMLV	Tymoviridae	84.3% (306/363)	79.5% (97/122)	84.1% (90/107)	*N. ceranae* ($p < 0.001$)	No

Prevalences of honey bee pathogens found in Belgian honey bee colonies, the relationships between these pathogens and the effect of the occurrence of each pathogen on colony winter losses.

*These data includes a subset of the samples (229), since 25% of the beekeepers did not provide data about winter losses of the monitored colonies.

doi:10.1371/journal.pone.0072443.t001

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Comprehensive Bee Pathogen Screening in Belgium Reveals *Crithidia mellificae* as a New Contributory Factor to Winter Mortality

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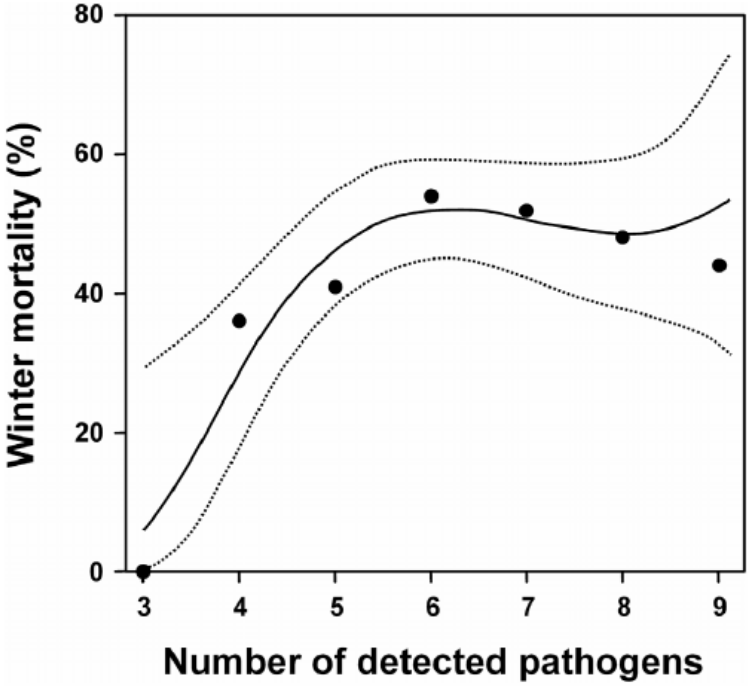

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disadvantages of molecular diagnostics:

cfr. the *Nosema ceranae*-case: first discovery

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Journal of Invertebrate Pathology 92 (2006) 93–95
Journal of INVERTEBRATE PATHOLOGY
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Short communication

Nosema ceranae, a new microsporidian parasite in honeybees in Europe

Mariano Higes [a,*], Raquel Martín [a], Aránzazu Meana [b]

[a] *Centro Apícola Regional, Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha, Marchamalo, 19180 Guadalajara, Spain*
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Original article

A *Nosema ceranae* isolate from the honeybee *Apis mellifera

Wei-Fone HUANG[a], Jing-Hao JIANG[a], Yue-Wen CHEN[b], Chung-Hsiung WANG[a]

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[b] Department of Animal Science, National I-Lan University, I-Lan 260, Taiwan

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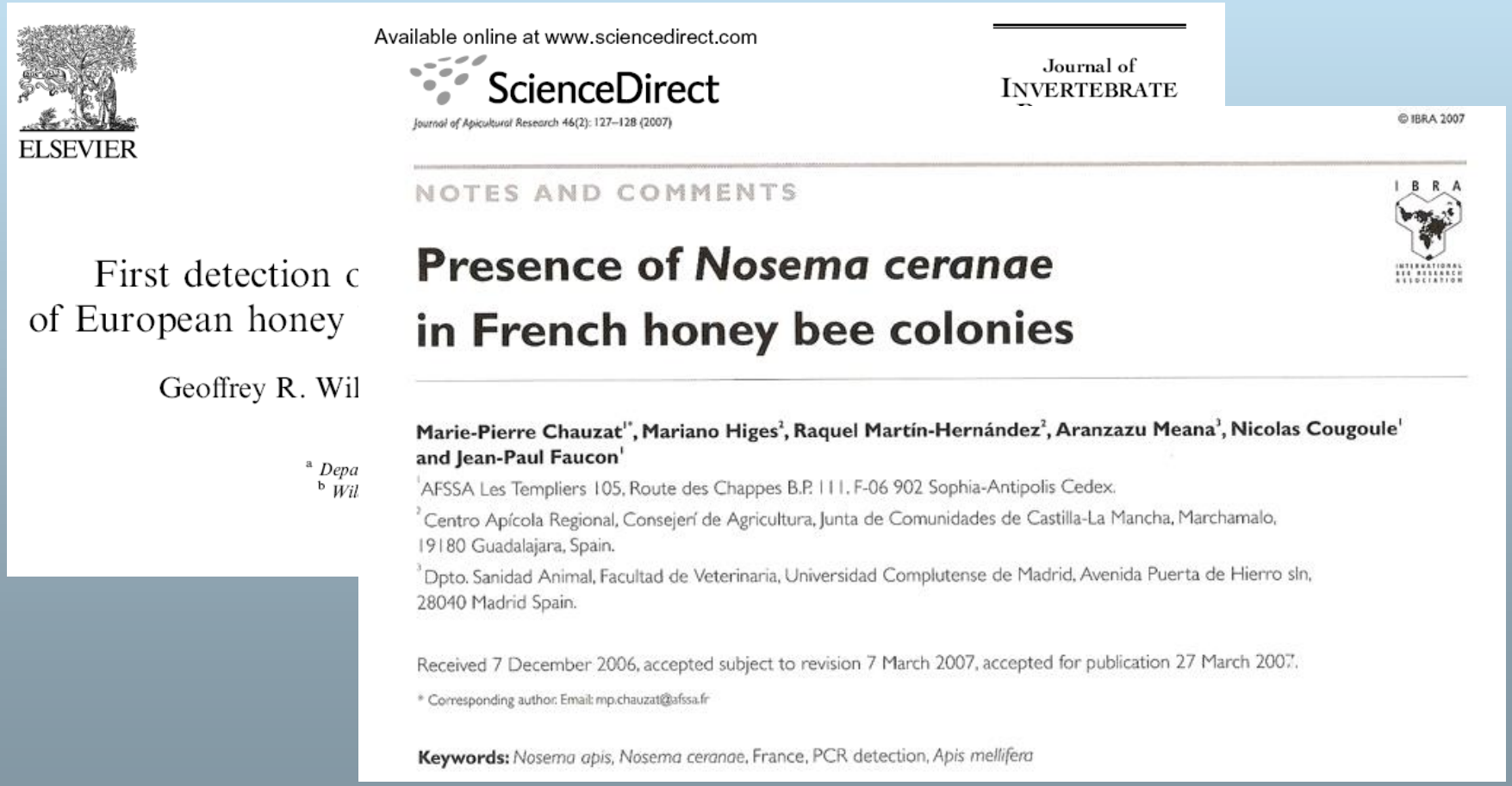
disadvantages of molecular diagnostics:

cfr. the *Nosema ceranae*-case: widespread



ELSEVIER

First detection c
of European honey

Geoffrey R. Wil

[a] Depa
[b] Wil

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Journal of Apicultural Research 46(2): 127–128 (2007)

Journal of
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NOTES AND COMMENTS

IBRA

Presence of *Nosema ceranae* in French honey bee colonies

Marie-Pierre Chauzat[1*], Mariano Higes[2], Raquel Martín-Hernández[2], Aranzazu Meana[3], Nicolas Cougoule[1] and Jean-Paul Faucon[1]

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* Corresponding author. Email: mp.chauzat@afssa.fr

Keywords: *Nosema apis*, *Nosema ceranae*, France, PCR detection, *Apis mellifera*

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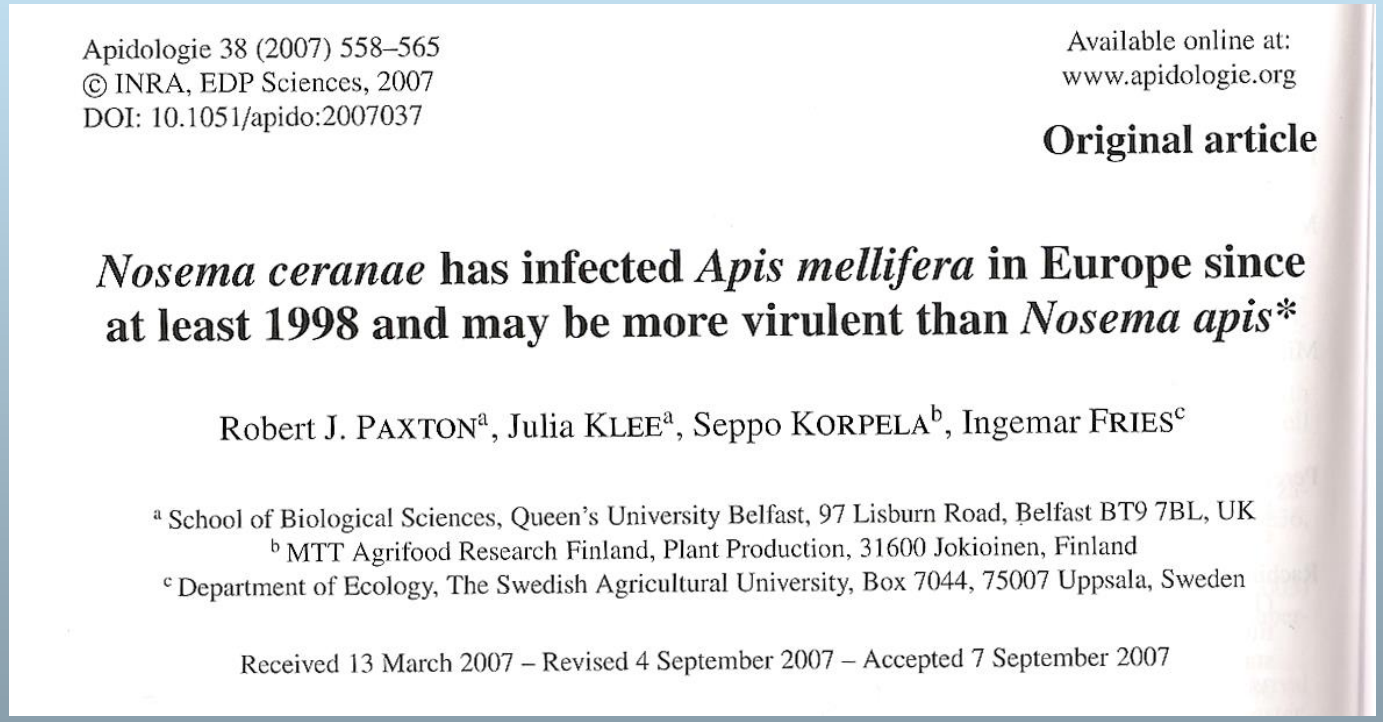
disadvantages of molecular diagnostics:

cfr. the *Nosema ceranae*-case: retrospective studies



Apidologie 38 (2007) 558–565
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DOI: 10.1051/apido:2007037

Available online at:
www.apidologie.org

Original article

Nosema ceranae has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis**

Robert J. PAXTON[a], Julia KLEE[a], Seppo KORPELA[b], Ingemar FRIES[c]

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'you only find what you are looking for'

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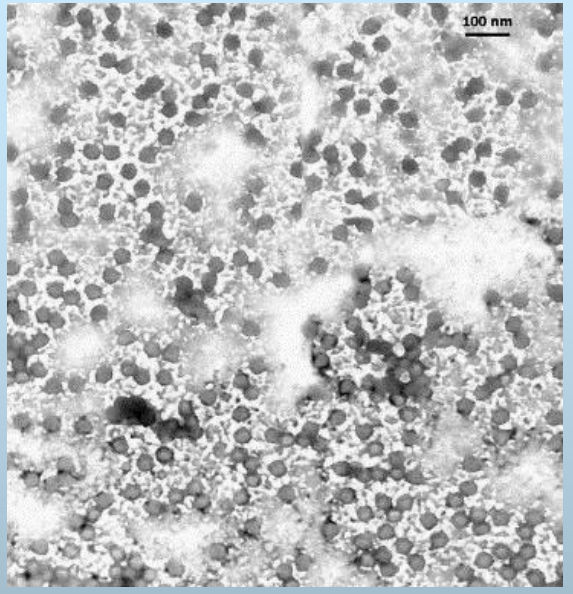
what about rapidly evolving pathogens?

bee viruses

ABPV – KBV – IAPV

DWV – KV – VDV



what about other diseases?

environmental microbiology reports

***Apicystis bombi* (Apicomplexa: Neogregarinorida) parasitizing *Apis mellifera* and *Bombus terrestris* (Hymenoptera: Apidae) in Argentina**

Santiago Plischuk[1,*], Ivan Meeus[2], Guy Smagghe[2], Carlos E. Lange[1]

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developing molecular tools with reduced specificity:

Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers

I. Meeus[1,2], D.C. de Graaf[2], K. Jans[3] and G. Smagghe[1]

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looking at all the microbes

tsI, also has $(\phi_I, \psi_I)_{ts} = (0°, 70°)$.

By probing the HJ dynamics in response to pulling forces in three different directions, we mapped the location of the transition states in the two-dimensional (2D) reaction landscape and deduced the global structure of the transient species populated during the HJ conformational changes. Our simplest model envisions a shallow minimum between the two transition states, depicted as the open structure (Fig. 3A and 3D), but it is also possible that a continuum of conformations exist, spanning from *ts*I and *ts*II with nearly identical free energies, instead of having a single well-defined minimum.

The development reported here expands on the current arsenal of hybrid single-molecule techniques combining force and other observables (*8*, *25*–*27*). Unlike DNA or RNA hairpins, where forces on the order of 15 pN are necessary to induce mechanical unzipping (*10*, *11*), the conformations of HJs could be biased at 0.5 pN or lower. The lever-arm effect makes it unlikely that a purely mechanical tool could have probed the force effect on HJ conformations, because if the arms are lengthened to magnify the distance change, the force effect will occur at even lower forces. FRET can also report on vectors other than the end-to-end distances, which we exploited here by pulling on *XR*, *HR*, or *BR* arms while simultaneously measuring the same HB vector by FRET, which led to the 2D mapping of reaction landscapes. Our method is readily applicable to other nucleic acids systems and their interaction with proteins and enzymes, and with the advent of new orthogonal labeling techniques, should be extendable

A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder

Diana L. Cox-Foster,[1] Sean Conlan,[2] Edward C. Holmes,[3,4] Gustavo Palacios,[2] Jay D. Evans,[5] Nancy A. Moran,[6] Phenix-Lan Quan,[2] Thomas Briese,[2] Mady Hornig,[2] David M. Geiser,[7] Vince Martinson,[8] Dennis vanEngelsdorp,[1,9] Abby L. Kalkstein,[1] Andrew Drysdale,[2] Jeffrey Hui,[2] Junhui Zhai,[2] Liwang Cui,[1] Stephen K. Hutchison,[10] Jan Fredrik Simons,[10] Michael Egholm,[10] Jeffery S. Pettis,[5] W. Ian Lipkin[2]*

In colony collapse disorder (CCD), honey bee colonies inexplicably lose their workers. CCD has resulted in a loss of 50 to 90% of colonies in beekeeping operations across the United States. The observation that irradiated combs from affected colonies can be repopulated with naive bees suggests that infection may contribute to CCD. We used an unbiased metagenomic approach to survey microflora in CCD hives, normal hives, and imported royal jelly. Candidate pathogens were screened for significance of association with CCD by the examination of samples collected from several sites over a period of 3 years. One organism, Israeli acute paralysis virus of bees, was strongly correlated with CCD.

Methods for cloning nucleic acids of microbial pathogens directly from clinical and environmental specimens afford unprecedented opportunities for pathogen discovery and surveillance. Subtractive cloning, polymerase chain reaction (PCR), and DNA microarrays have implicated previously unknown pathogens as the etiological agents of several acute and chronic diseases. Here, we describe the application of unbiased high-throughput pyrosequencing technology (*1*) in the characterization of the microflora associated with *Apis mellifera* in a search for the cause of colony collapse disorder (CCD).

CCD is characterized by the rapid loss from a colony of its adult bee population (*2*–*4*). No dead adult bees are found inside or in close proximity to the colony. At the final stages of collapse, a queen is attended only by a few newly emerged adult bees. Collapsed colonies often have considerable capped brood and food reserves. The phenomenon of CCD was first reported in 2006;

OPEN ACCESS Freely available online

PLoS one

Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, *Nosema*, and *Crithidia*

Charles Runckel[1,2☯], Michelle L. Flenniken[3☯], Juan C. Engel[4], J. Graham Ruby[1,2], Donald Ganem[1,2], Raul Andino[3], Joseph L. DeRisi[1,2*]

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what about other causes of death - bad performence?

over-exposure to pesticides

GC-MS analysis of pesticides in case of intoxication: 290 €

LIMITATIONS OF FINGERPRINTING

what about other causes of death - bad performence?

food shortage - incomplete diet

BEECLINIC

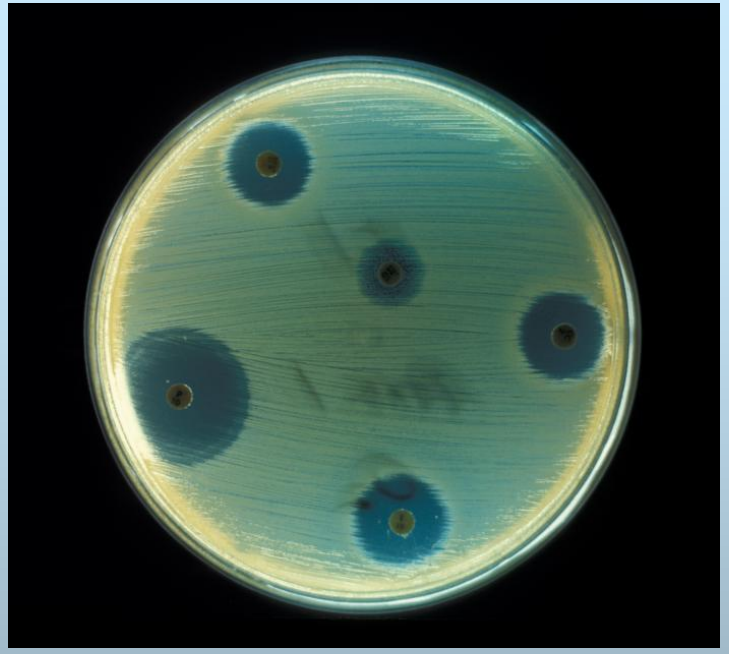
listen to what our body tells us!

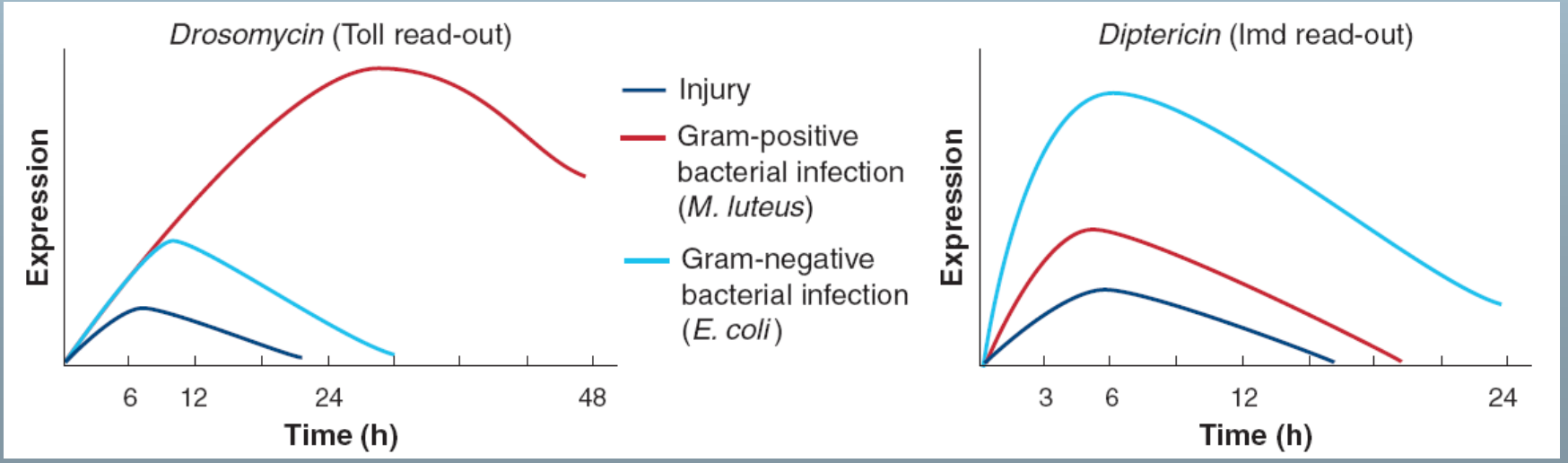
fever < inflammation (stress indicator)

BEECLINIC

insect immunity:
humoral response
antimicrobial peptides
= immune end product
= stress indicators

1 2 3 4 5 6 7





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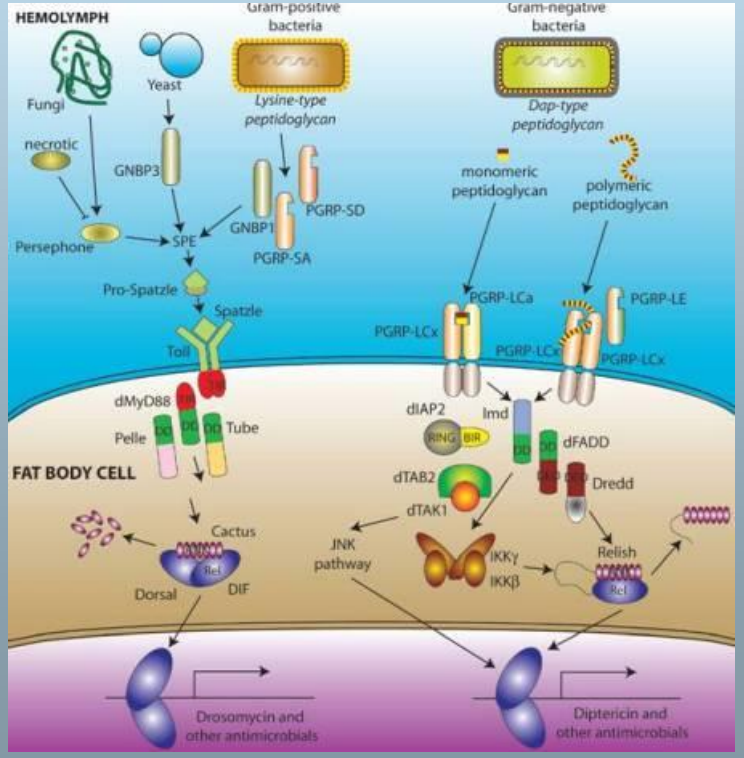
insect immunity:
humoral response
signalling pathway
= stress indicators

8 9 10 11 12 13 14
15 16 17 18 19 20 21

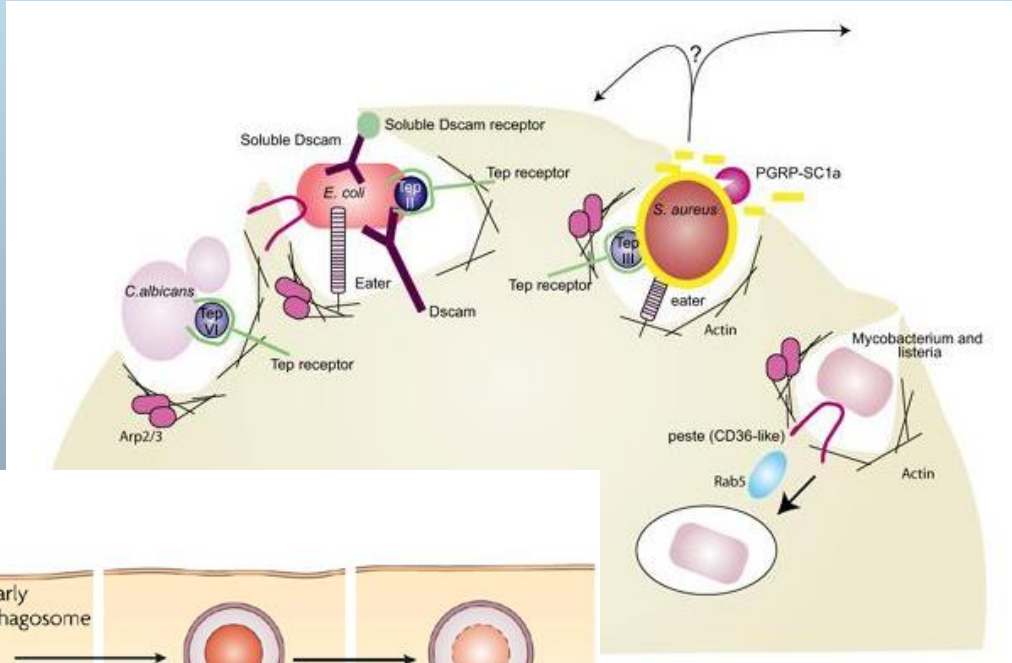


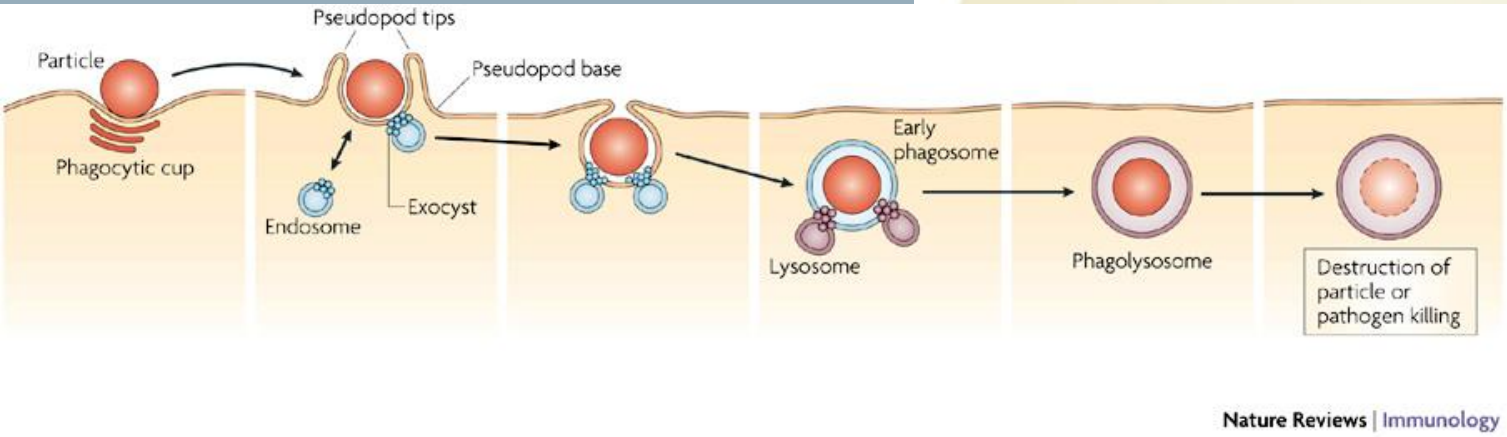
BEECLINIC

insect immunity:
cellular response
fagocytosis
receptors
= stress indicators

22 23 24 25 26 27 28

29 30 31 32 33 34 35





BEECLINIC

detoxification:

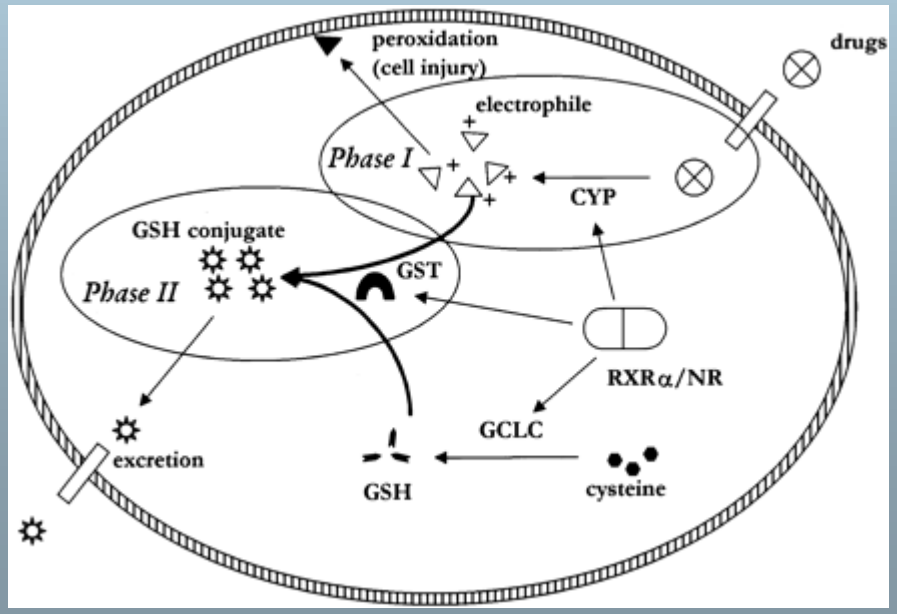
honey bee genome project

30-50% fewer genes encoding

- carboxylesterase
- cytochrome P450
- glutathione S-transferase

= toxicological stress indicators

1 2 3 4 5 6 7



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malnutrition:

cfr. J. Van der Steen

measuring colony fitness

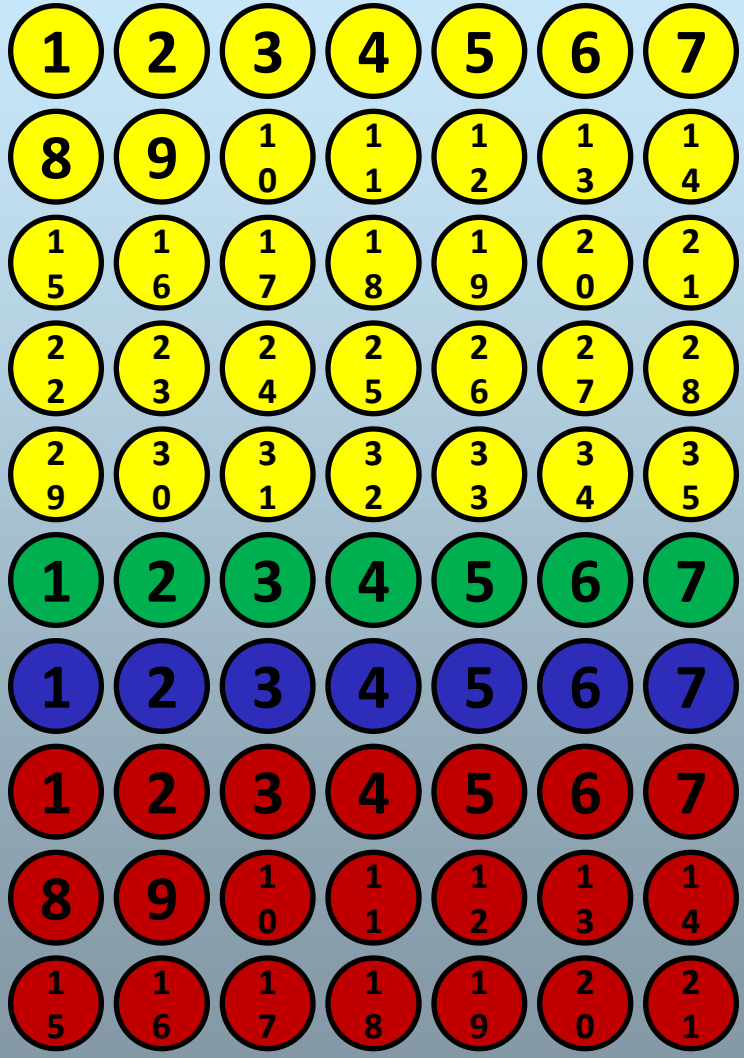
< mean hemolymph vitellogenin concentration

(influenced by *Varroa destructor*, discontinuous pollen flow, low diversity of pollen)

= nutritional stress indicators

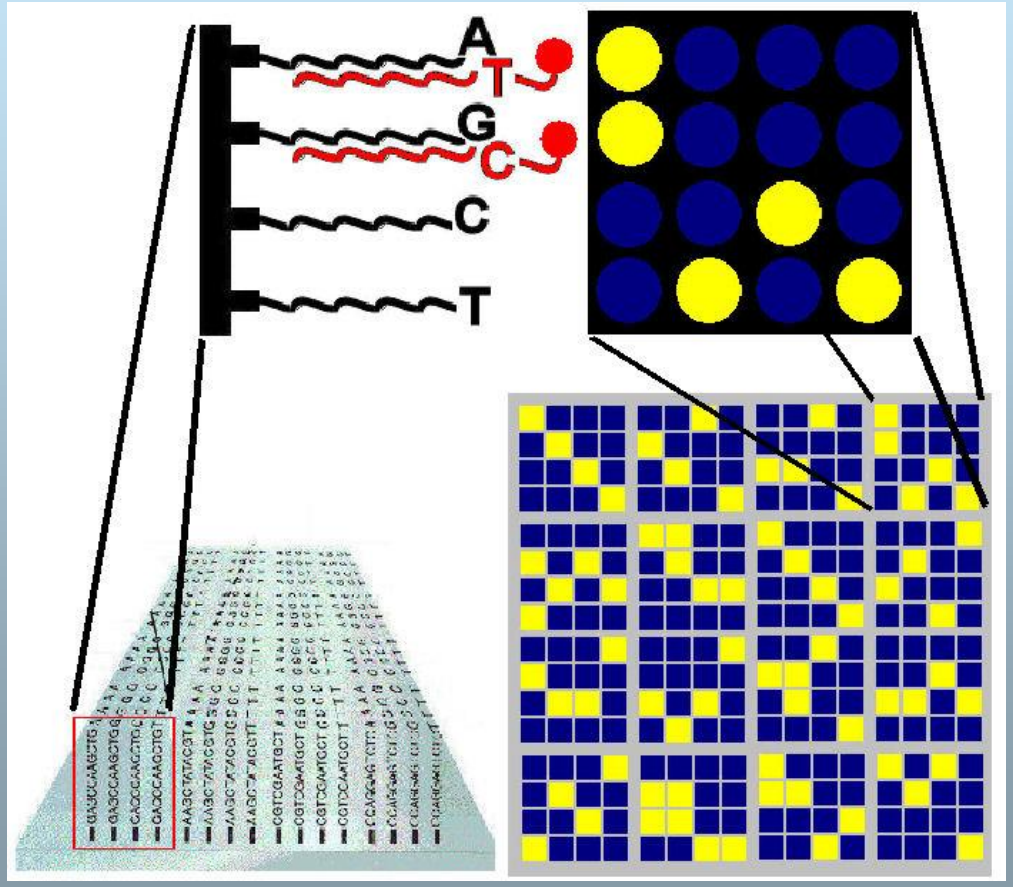
BEECLINIC

DNA-chip technology:
probes against each
stress indicator
+
every known bee pathogen



BEECLINIC

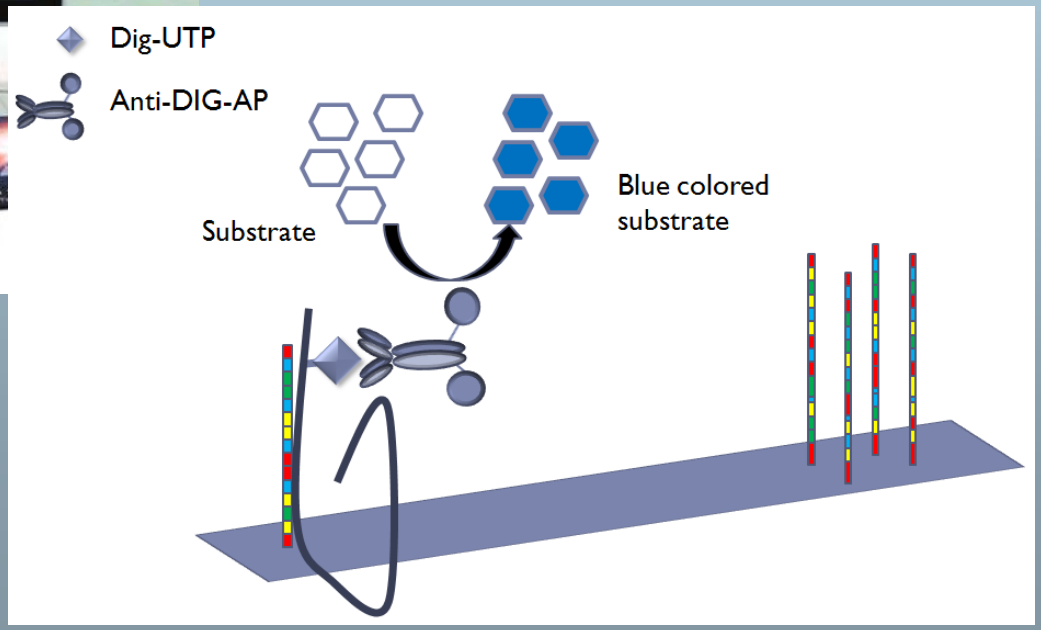
DNA-chip technology:



BEECLINIC

making DNA-chip technology accessible:

ArrayIT® SpotWave ™ Colorimetric Microarray scanner



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DNA chip first prototype:

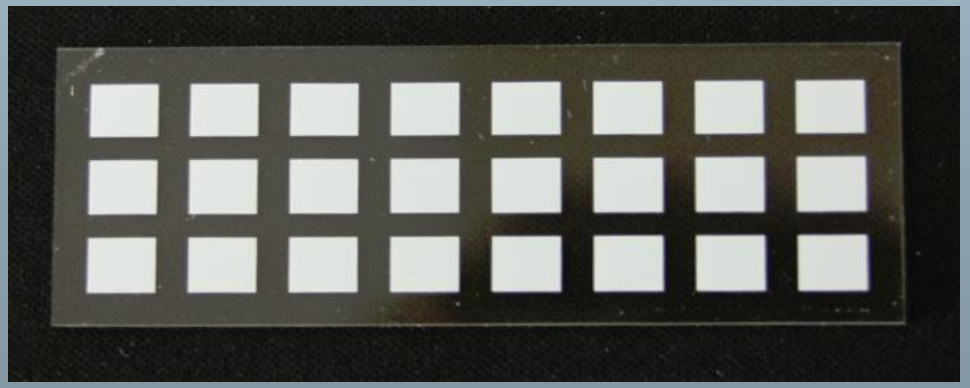
- printing on nylon coated glass slides
- multi-well cassette: multiplex 24 samples

DNA chip second prototype:

- nylon cut in 24 squares

> covering 1 DNA chip each

>> better sealing of multi-well cassette



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selection of targets:

- simultaneous measurement of honeybee genes and pathogens
- cfr. Evans, 2006:
  - quantitative-PCR array
  - 48 targets

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ScienceDirect

Journal of Invertebrate Pathology 93 (2006) 135–139

Journal of INVERTEBRATE PATHOLOGY

www.elsevier.com/locate/yjipa

Short communication

Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease

Jay D. Evans *

USDA-ARS Bee Research Laboratory, BARC-East Bldg., 476 Beltsville, MD 20705, USA

BEECLINIC

targets :

R, pathogen recognition

E, immune end product

D, developmental genes

S, immune signalling pathway

C, controls

P, pathogens

Locus	Cat	Locus	Cat	Locus	Cat	Locus	Cat	Locus	Cat
BglucA	R	Am53C8	D	Dorsal-2	S	Spaetzle	S	Dscam	E
PGRP9710	R			Dredd	S	Tab	S	EGFlikeA	E
PGRPLC710	R	Toll	S	hemipterous	S	Tak1	S	Hymenopt	E
PGRPSC2505	R	Basket	S	hopscotch	S			Lys-1	E
PGRPSC4300	R	Cactus-1	S	Imd	S	Abaecin	E	Lys-2	E
		Cactus-2	S	Kenny	S	AmPPO	E	Lys-3l	E
RPL8	C	Domeless	S	Myd88	S	Apidaec	E	PPOact	E
RPS5	C	Dorsal-1	S	Perseph	S	Defensin2	E	TEP7	E
				Relish	S	Defensin1	E	TEPA	E

Locus	Cat
A. apis	P
M. pluton	P
N. apis	P
chitinA	P
germSA	P
PIS18	P
Bact16S	P

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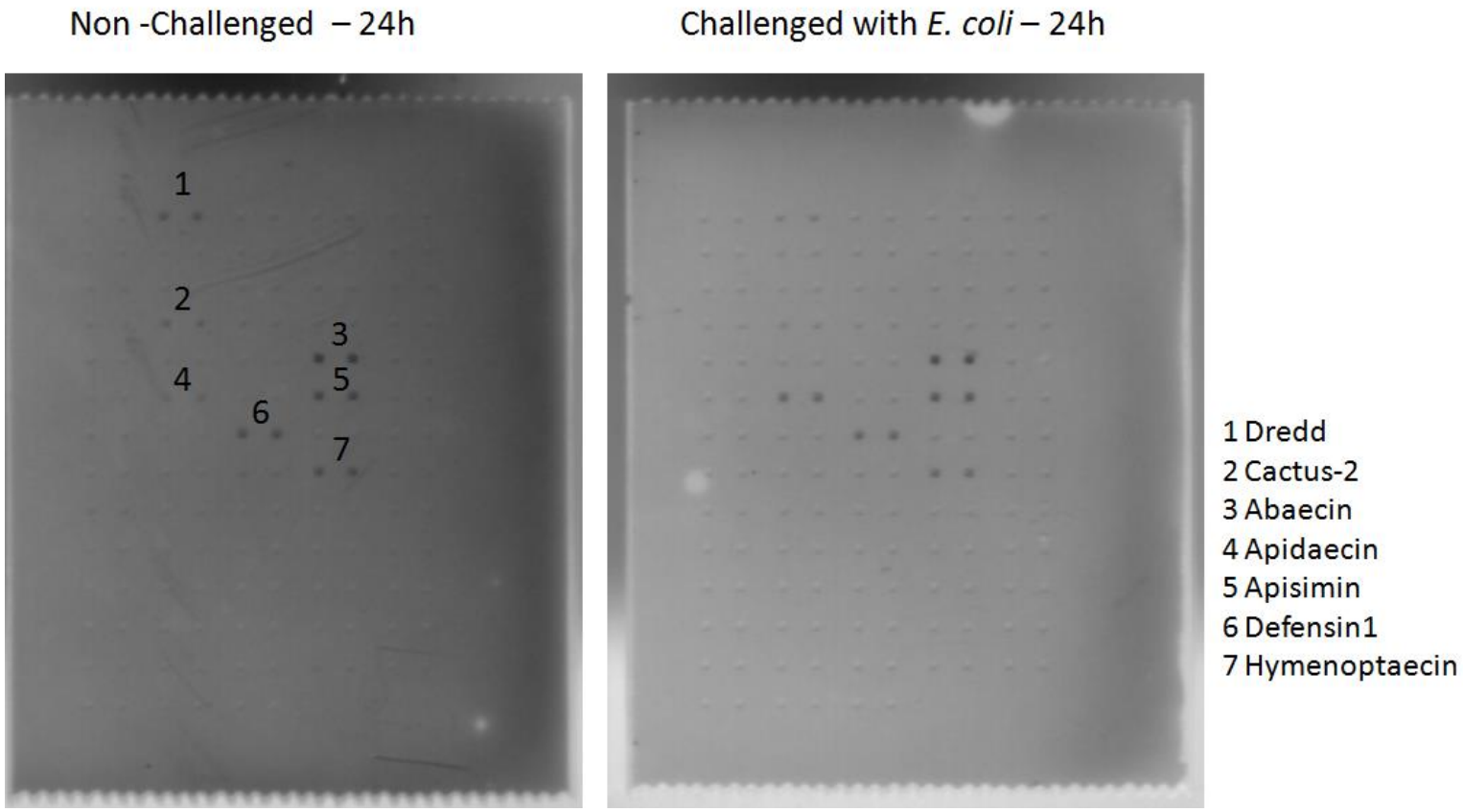
DNA chip second prototype:

- first run:



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DNA chip third prototype:

- extension of target number = 110 (in double)

pathogen: 20
pathogen recognition: 4
immune signalling pathway: 18
immune end product: 16
pesticide exposure: 9
nutritional stress: 8
varroa infestation: 10
nosema infestation: 9
varroa tolerance/QTL: 1
nosema tolerance/QTL: 2
transition summer/winter bee: 6
transition fourager bee: 1
transition sterile/fertile worker bee: 1
housekeeping genes: 3
negative ctl: 2

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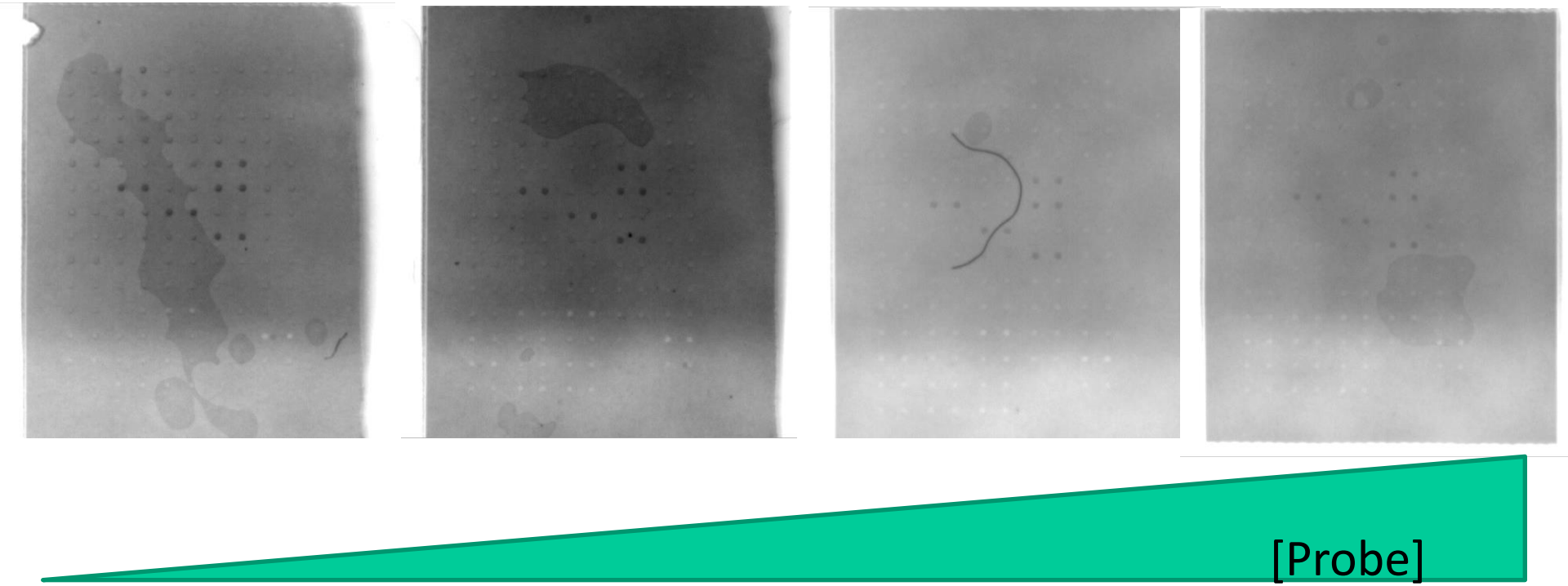
quantificantion of spot intensity (TIFF-files):
< MAPIX software

optimization:



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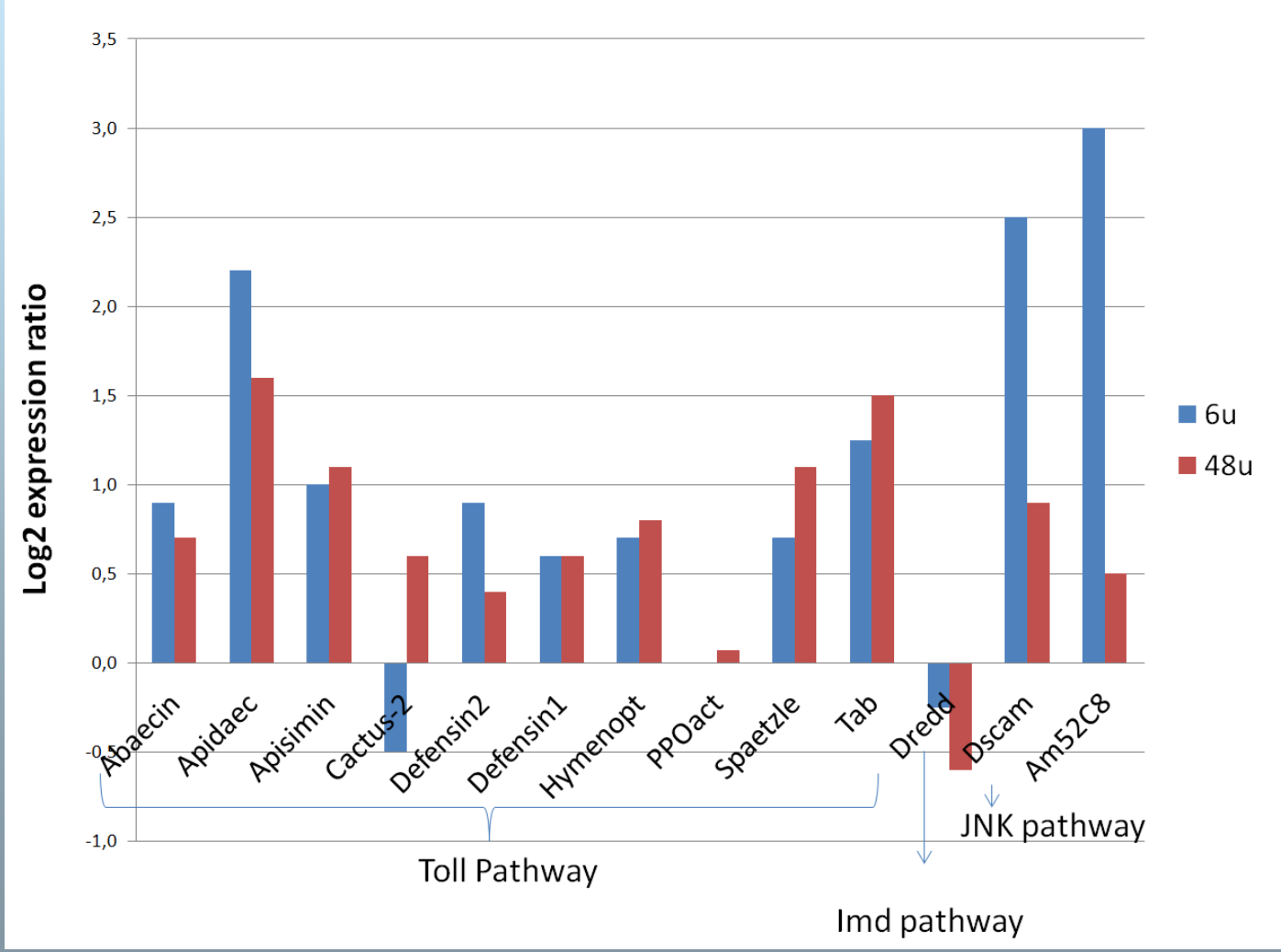
E. coli infection:



WORKING WITH BEECLINIC

- 2 week sampling:
  - 5 educative apiaries
  - 10 colonies each
  - started at July 2011
- objectives:
  - unique samples

x transition summer/winter
x just before colony collapse

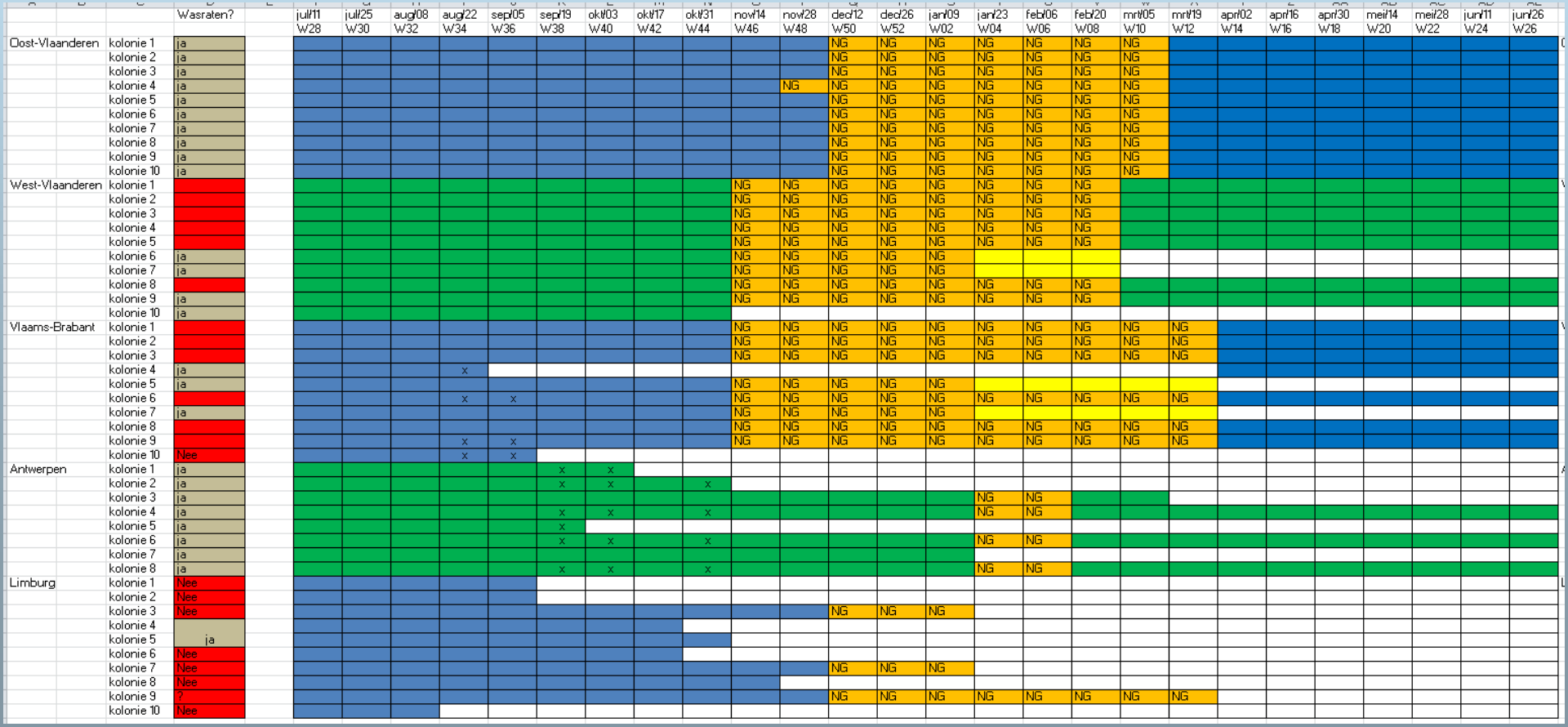
insight in moment of collapse

BeeClinic on 22 selected samples

insight in cause of collapse

WORKING WITH BEECLINIC

- results:
winter losses in 27/48 colonies (56%)
death: late summer/autumn



		Wasraten?	jul/11	jul/25	aug/08	aug/22	sept/05	sept/19	okt/03	okt/17	okt/31	nov/14	nov/28	dec/12	dec/26	jan/09	jan/23	feb/06	feb/20	mrt/05	mrt/19	apr/02	apr/16	apr/30	mei/14	mei/28	jun/11	jun/26
			W28	W30	W32	W34	W36	W38	W40	W42	W44	W46	W48	W50	W52	W02	W04	W06	W08	W10	W12	W14	W16	W18	W20	W22	W24	W26
Oost-Vlaanderen	kolonie 1	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 2	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 3	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 4	ja											NG	NG	NG	NG	NG	NG	NG	NG								
	kolonie 5	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 6	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 7	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 8	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 9	ja												NG	NG	NG	NG	NG	NG	NG								
	kolonie 10	ja												NG	NG	NG	NG	NG	NG	NG								
West-Vlaanderen	kolonie 1											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 2											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 3											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 4											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 5											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 6	ja										NG	NG	NG	NG	NG												
	kolonie 7	ja										NG	NG	NG	NG	NG												
	kolonie 8											NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 9	ja										NG	NG	NG	NG	NG	NG	NG	NG									
	kolonie 10	ja																										
Vlaams-Brabant	kolonie 1											NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 2											NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 3											NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 4	ja				x																						
	kolonie 5	ja										NG	NG	NG	NG	NG												
	kolonie 6					x	x					NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 7	ja										NG	NG	NG	NG	NG												
	kolonie 8											NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 9					x	x					NG	NG	NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 10	Nee				x	x																					
Antwerpen	kolonie 1	ja						x	x																			
	kolonie 2	ja						x	x		x																	
	kolonie 3	ja															NG	NG										
	kolonie 4	ja						x	x		x						NG	NG										
	kolonie 5	ja						x																				
	kolonie 6	ja						x	x		x						NG	NG										
	kolonie 7	ja																										
	kolonie 8	ja						x	x		x						NG	NG										
Limburg	kolonie 1	Nee																										
	kolonie 2	Nee																										
	kolonie 3	Nee												NG	NG	NG												
	kolonie 4																											
	kolonie 5	ja																										
	kolonie 6	Nee																										
	kolonie 7	Nee												NG	NG	NG												
	kolonie 8	Nee																										
	kolonie 9	?												NG	NG	NG	NG	NG	NG	NG	NG							
	kolonie 10	Nee																										

WORKING WITH BEECLINIC

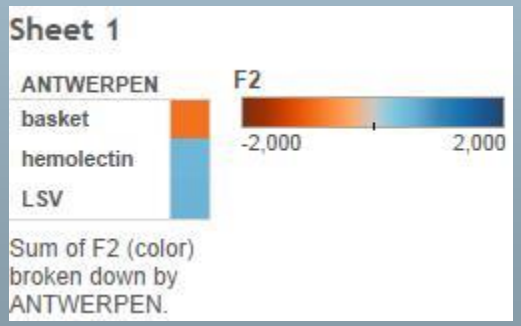
DNA-chip Antwerp:

few stress-indicators influenced

putative cause: *Nosema-Varroa*

heat maps:

imm-Nosema
Varroa (wound heling)
pathogen



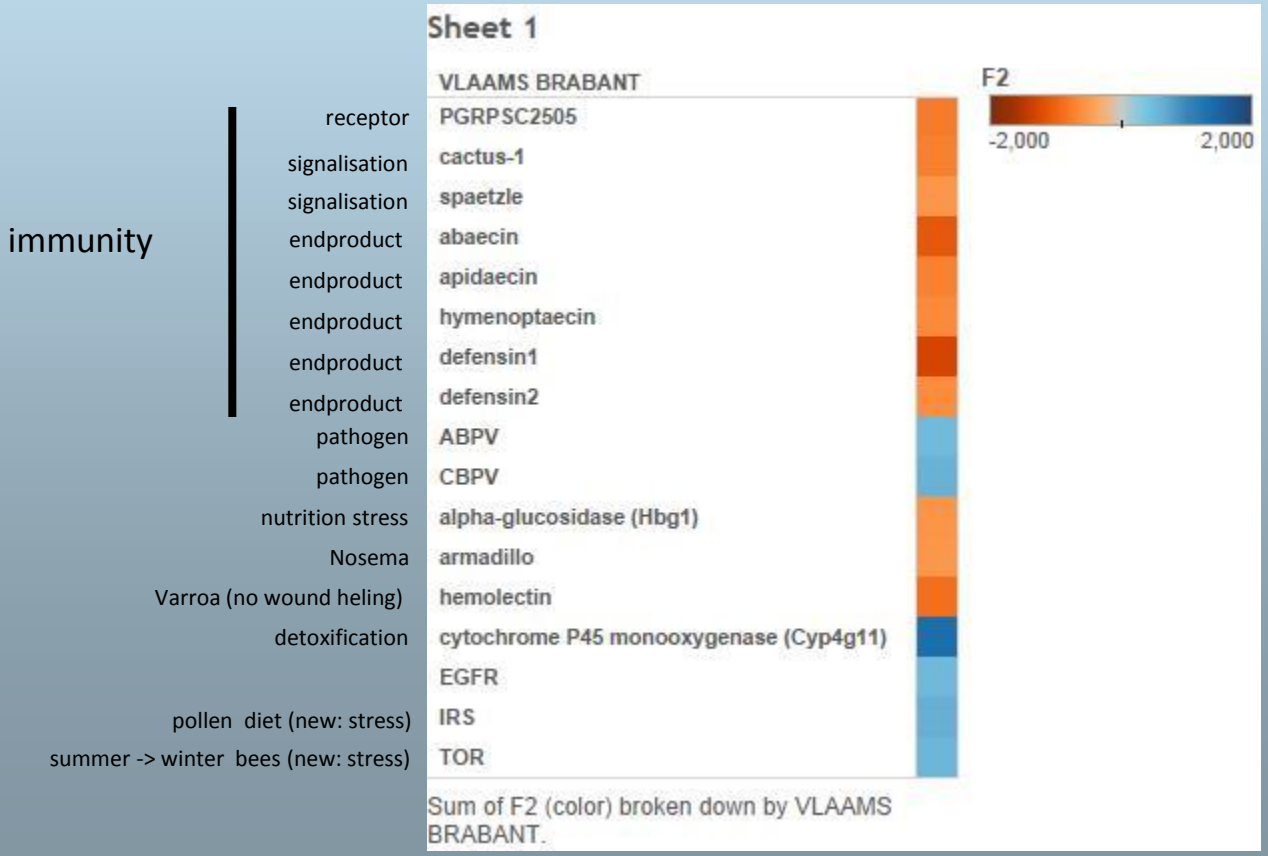
WORKING WITH BEECLINIC

DNA-chip Flemish Brabant:

many more stress-indicators influenced

putative cause: immunity down

pathogens, intoxication, nutrition???



CONCLUSIONS

development of an extension grade diagnostic tool
based on MLPA technology
focussing on bee viruses
= BeeDoctor

molecular fingerprinting tools have their limitation:
'you only find what you are looking for'

research grade diagnostics tool
based on colorimetric DNA chip technology
simultaneous measurement of honeybee genes and pathogens
= BeeClinic

first usage of BeeClinic shows
major geographic differences in causes of colony collapse