

# Kalkyngel

Annette Bruun Jensen

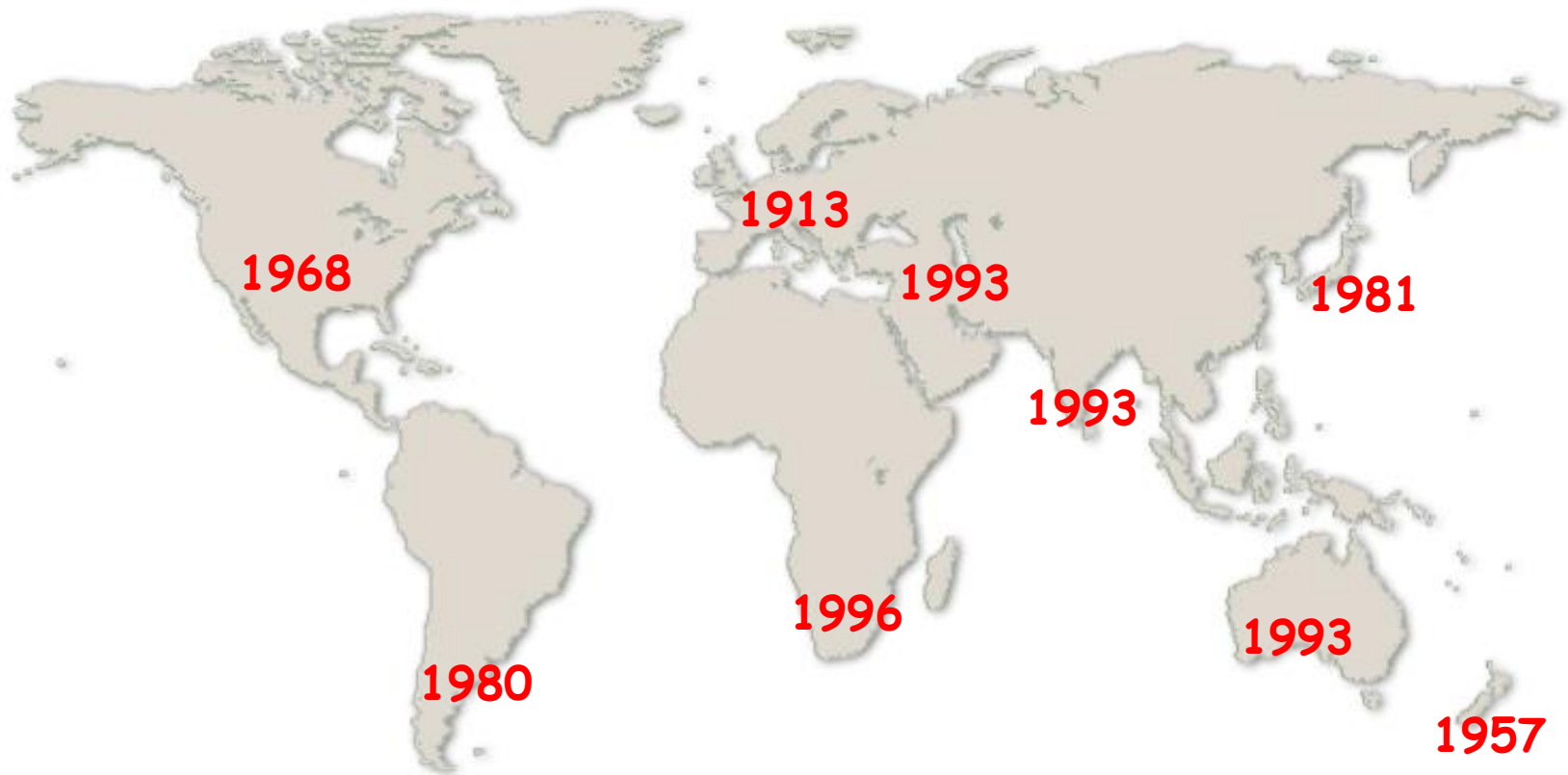
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Det Natur- og Biovidenskabelige Fakultet

KØBENHAVNS UNIVERSITET



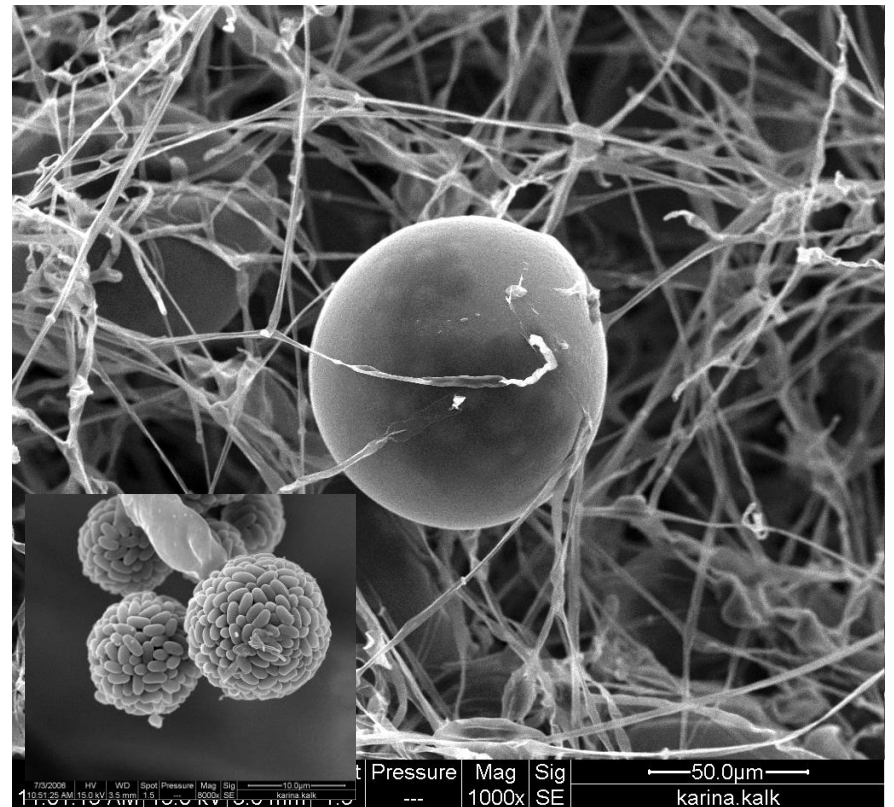
# Kalkyngel udbredt i hele verden



# Honningbi yngel-sygdomme

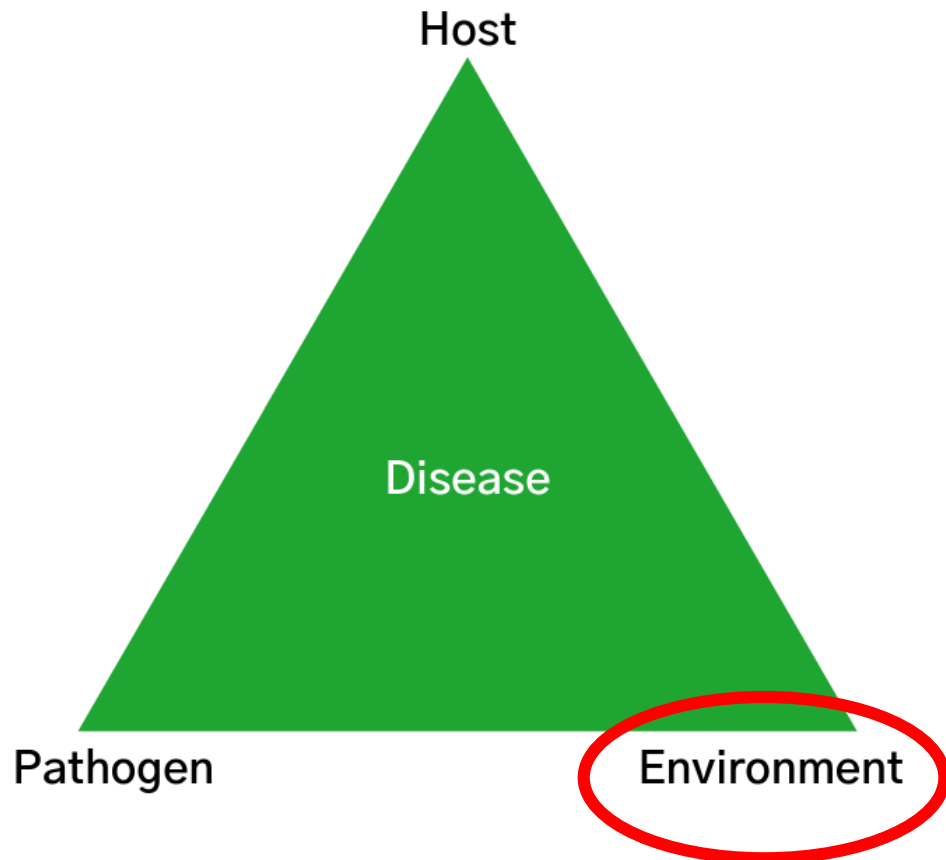
Pathogen	Taksonomisk gruppe	Stadie	Effekt på larven	Effekt på bifamilien
<b>Kalkyngel</b> <i>Ascospaera apis</i>	Svampe	Yngel	Lethal	Nedsat fitness
<b>Sækyngel</b> Sac Brood Virus	Virus	Yngel	Lethal	Nedsat fitness
<b>Europæisk bipest</b> <i>Melissococcus pluton</i>	Bakterie	Yngel	Lethal	Nedsat fitness
<b>Ondartet bipest</b> <i>Paenibacillus larvae</i>	Bakterie	Yngel	Lethal	<b>Lethal</b>

# Kalkyngel: svampen *Ascosphaera apis*





# Samspil mellem vært, pathogen og miljø



Kalkyngel – en forårsforkølelse

# Nedkøling af yngel giver mere kalkyngel

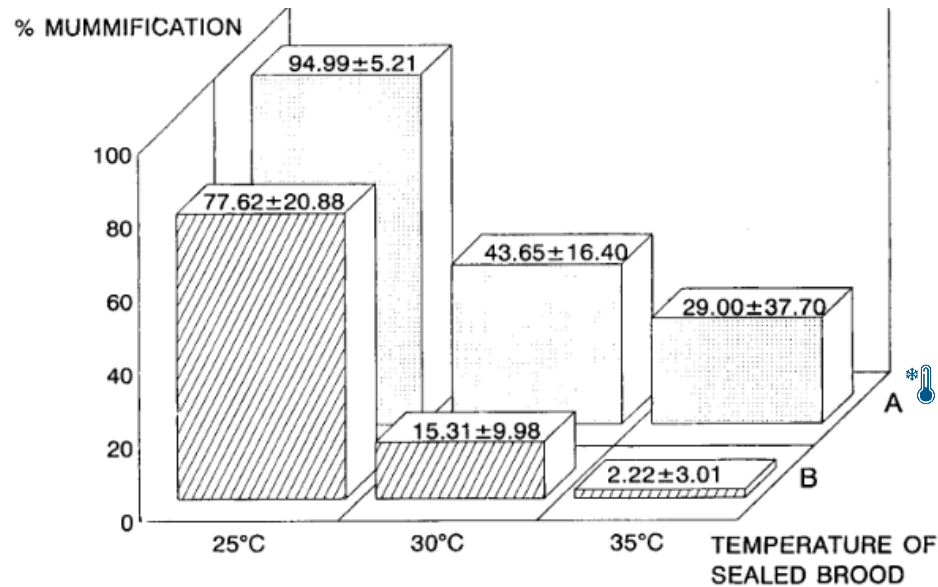
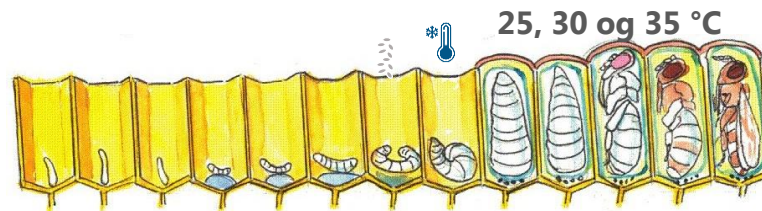
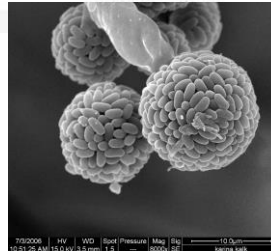
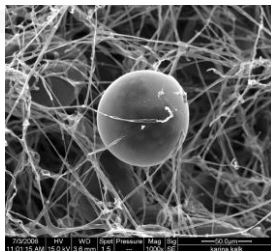
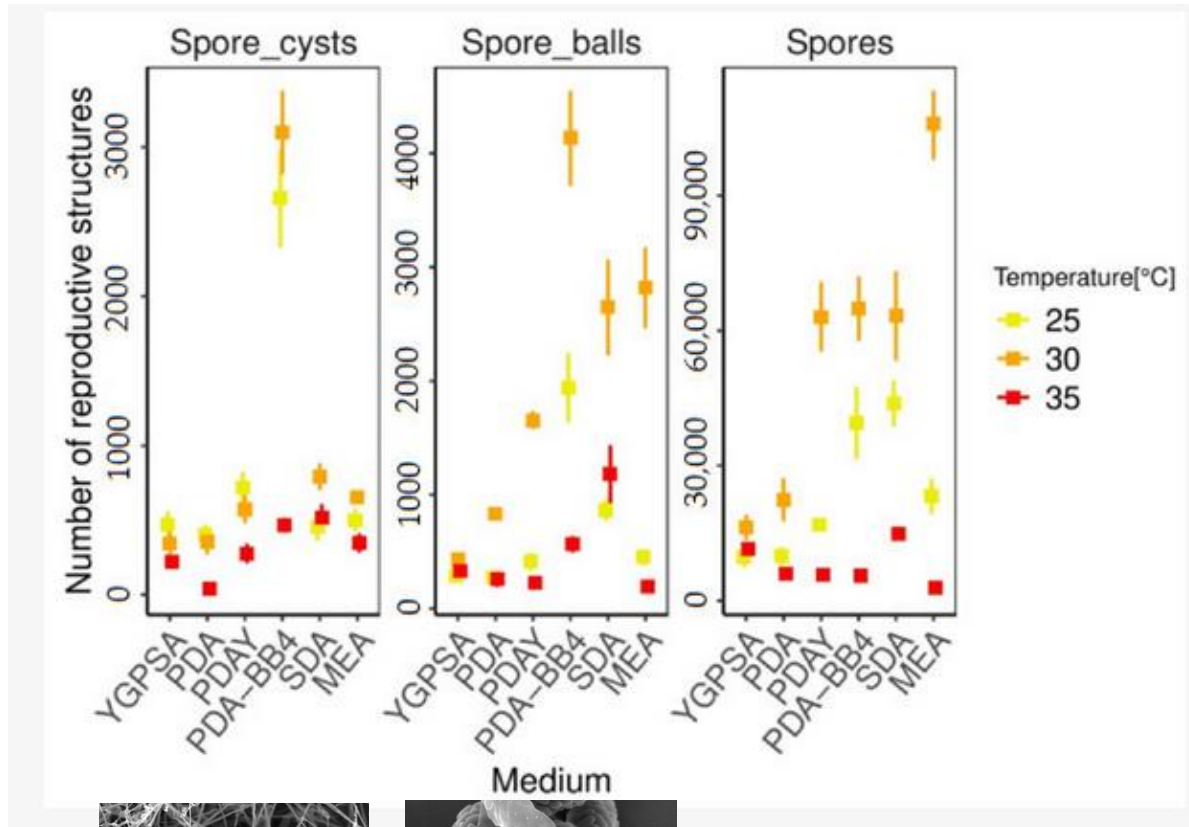


Fig 1. Percentage of mummification (mean + sd) at three temperatures: (A) with a cooling stress on L5 (18 °C 24 h before sealing) and (B) without the cooling stress. Data from table I.



# Produktion af infekitive spore og temperatur



Mráz et al 2021, Biology.



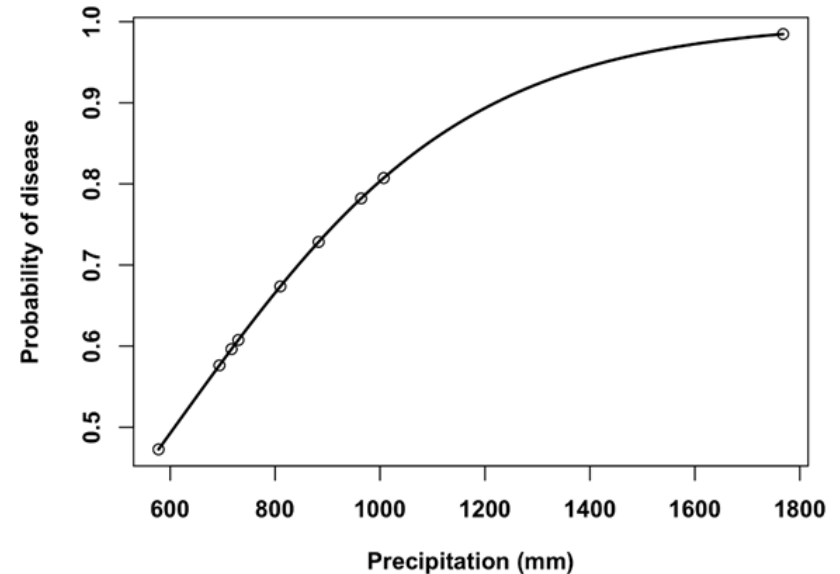
# Kalkyngel i Mexico - regn

Område	Kalkyngel infektion %	Climate area
Cocula	82.93	Warm subhumid
Sayula	73.08	Warm subhumid
Zacoalco	92.31	Warm subhumid
Tamazula	68.82	Warm subhumid
Tecalitlán	61.11	Warm subhumid
Tapalpa	78.38	Temperate subhumid
Gómez Farías	93.33	Temperate subhumid
Unión de Gpe.	60.00	Temperate subhumid
Zapotlán	57.14	Temperate subhumid

Table 3 Predictors of infection of honey bees by the fungus *Ascosphaera apis* in the logistic regression analysis (complete model)

Variables	Probability (Odds)	95 % CI	p
Temperature	*0.97	(0.69 - 1.37)	0.84
Precipitation	*3.38	(1.73 - 7.48)	<0.01
Height above the sea level	*0.97	(0.64 - 1.50)	0.89

\**Ascosphaera apis* colonies.



I alt 365 familier blev undersøgt om 74,1 % havde kalkyngel

# Kalkyngel – fugt og temperatur

- 1) Hyfevæksten stiger ved stigende temperaturer fra 25 to 35 °C
- 2) Optimal vandaktivitet for hyftevækst er 0.98
- 3) Vandaktiviteten på  $\geq 0.95$  er nødvendig for sporeproduktion



## Vandaktivitet

Rent vand = 1,00

Våd overflade  $\geq 0,90$

Fugtig overflade 0,65-0,85

# Kalkyngel – miljøet spiller en rolle

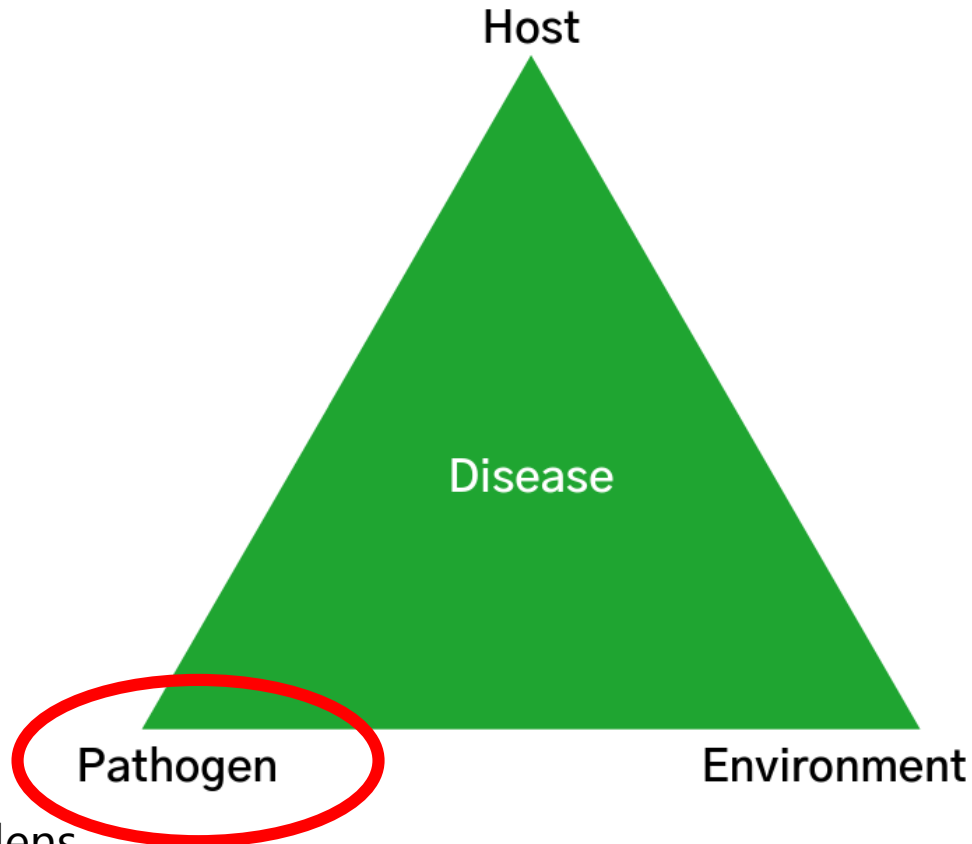


Temperatur og vand er vigtige for svampen

Placering af bistadet

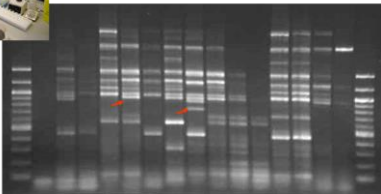
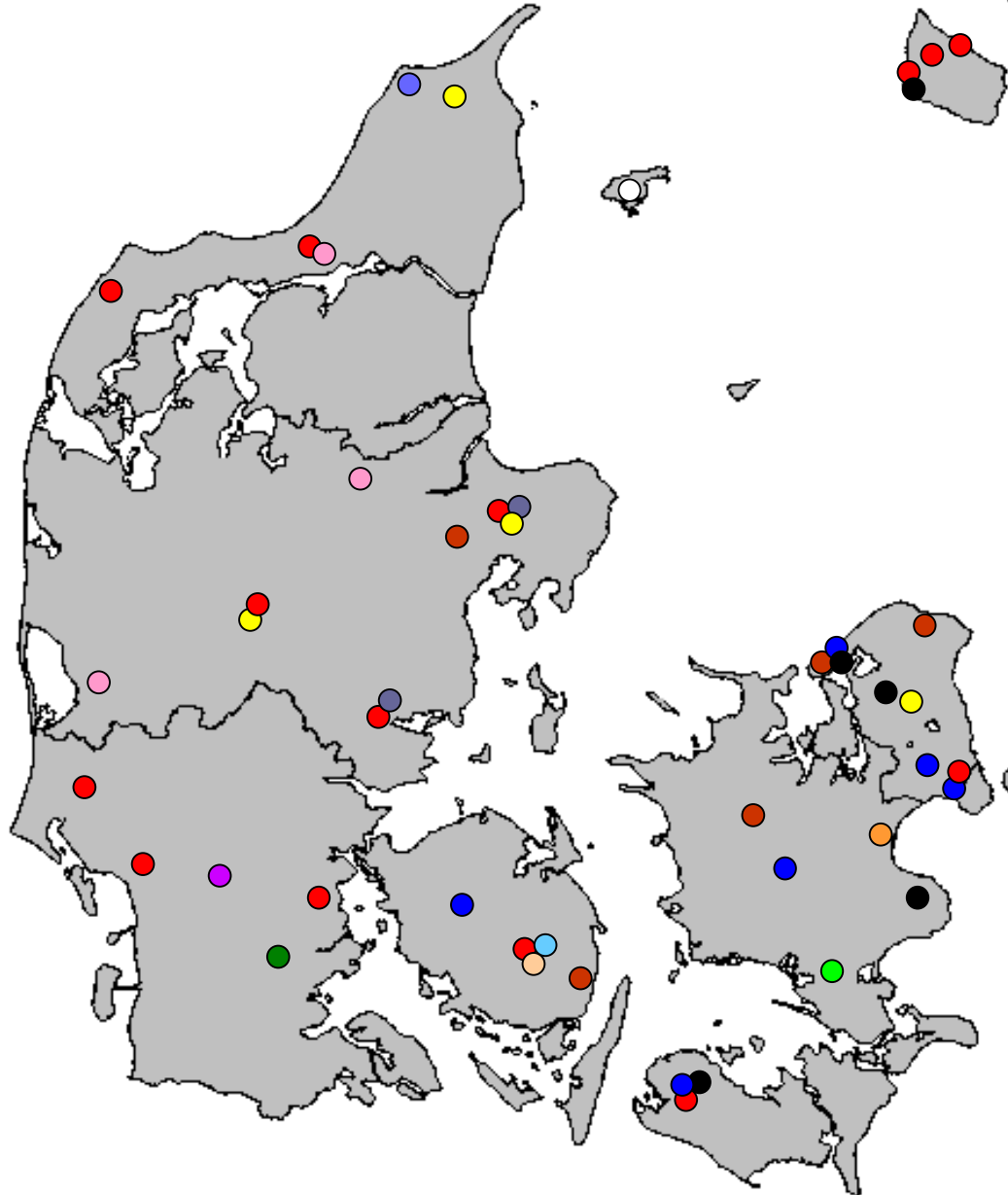
Bifamiliens fordeling af arbejder og yngel -  
især om foråret

# Samspil mellem vært, pathogen og miljø



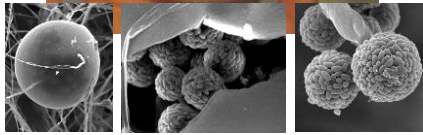
Forskellig virulens

# Genetisk variation i kalkyngel

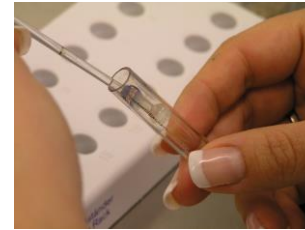
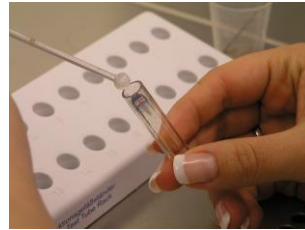


# Smitte material

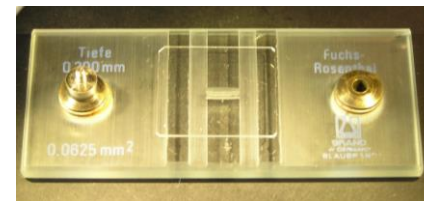
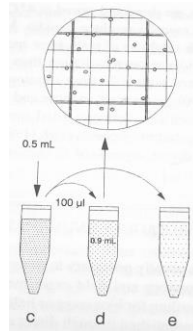
Agarplade/sorte mumier



Sporene frigøres

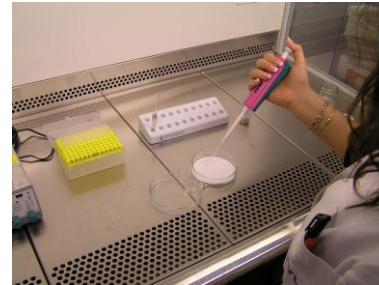
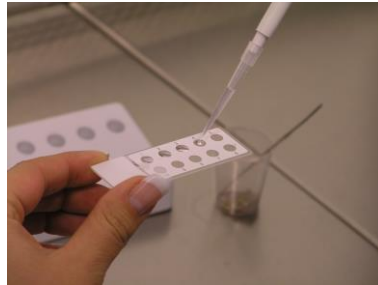


Koncentration af sporene



# Spore spirings test

Sporene blandes med flydende vækstmedie



Sporene tilsættes CO<sub>2</sub>

Inkuberes i 24 timer

Mikroskoperes



Spore spire  
procent optælles

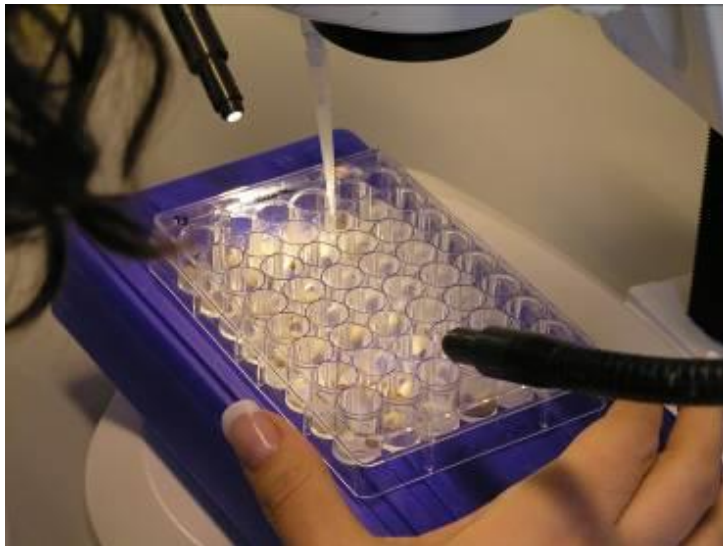


# Bioassay - Feltarbejde





## *In vitro* opdræt af honningbilarver



Mad:

50% gele Royal

6% glucose

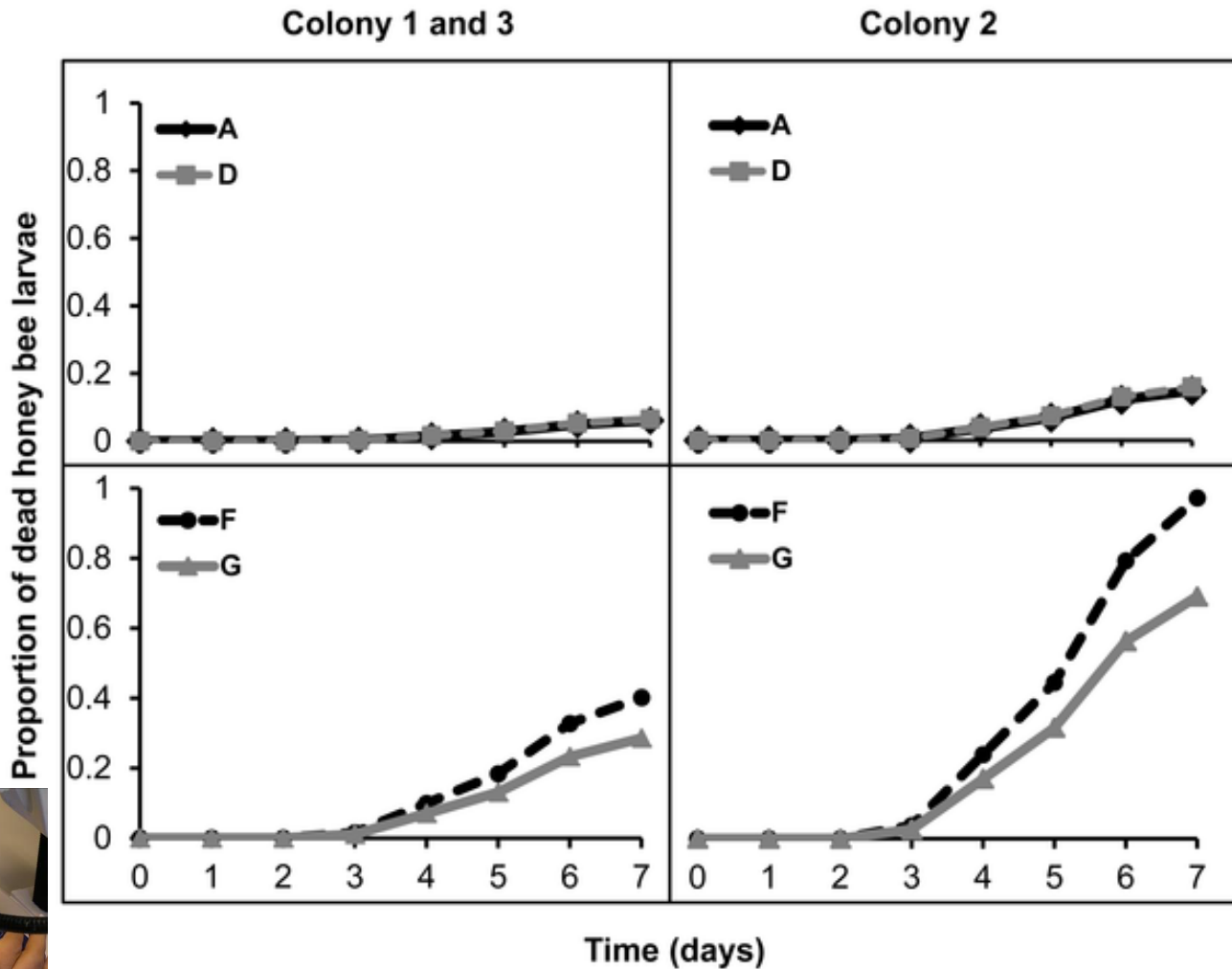
6% fruktose

Temperatur 34°C

Høj luftfugtighed



# Forskell i virulens af fire svampe isolater



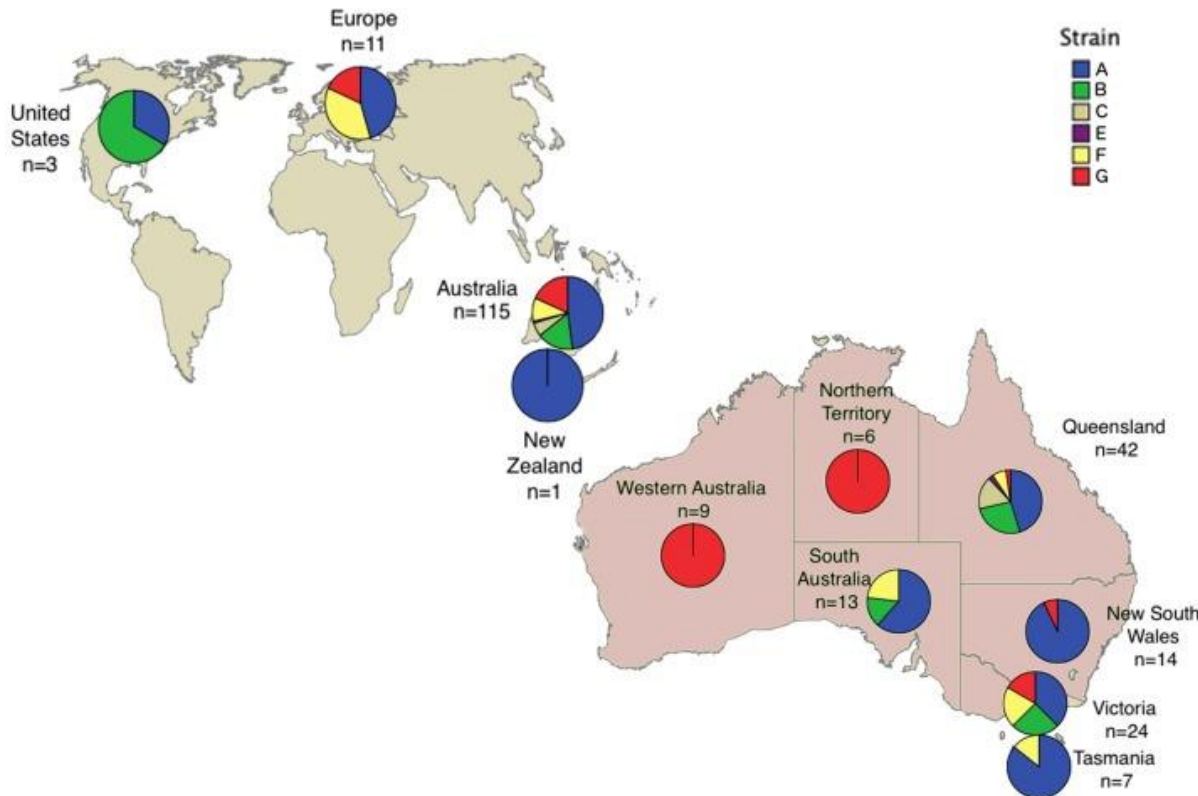
Cumulative proportions of honeybee larvae dead from chalkbrood disease after exposure to the four *Ascosphaera apis* strains A, D, F and G.

# Genetisk variation i kalkyngel



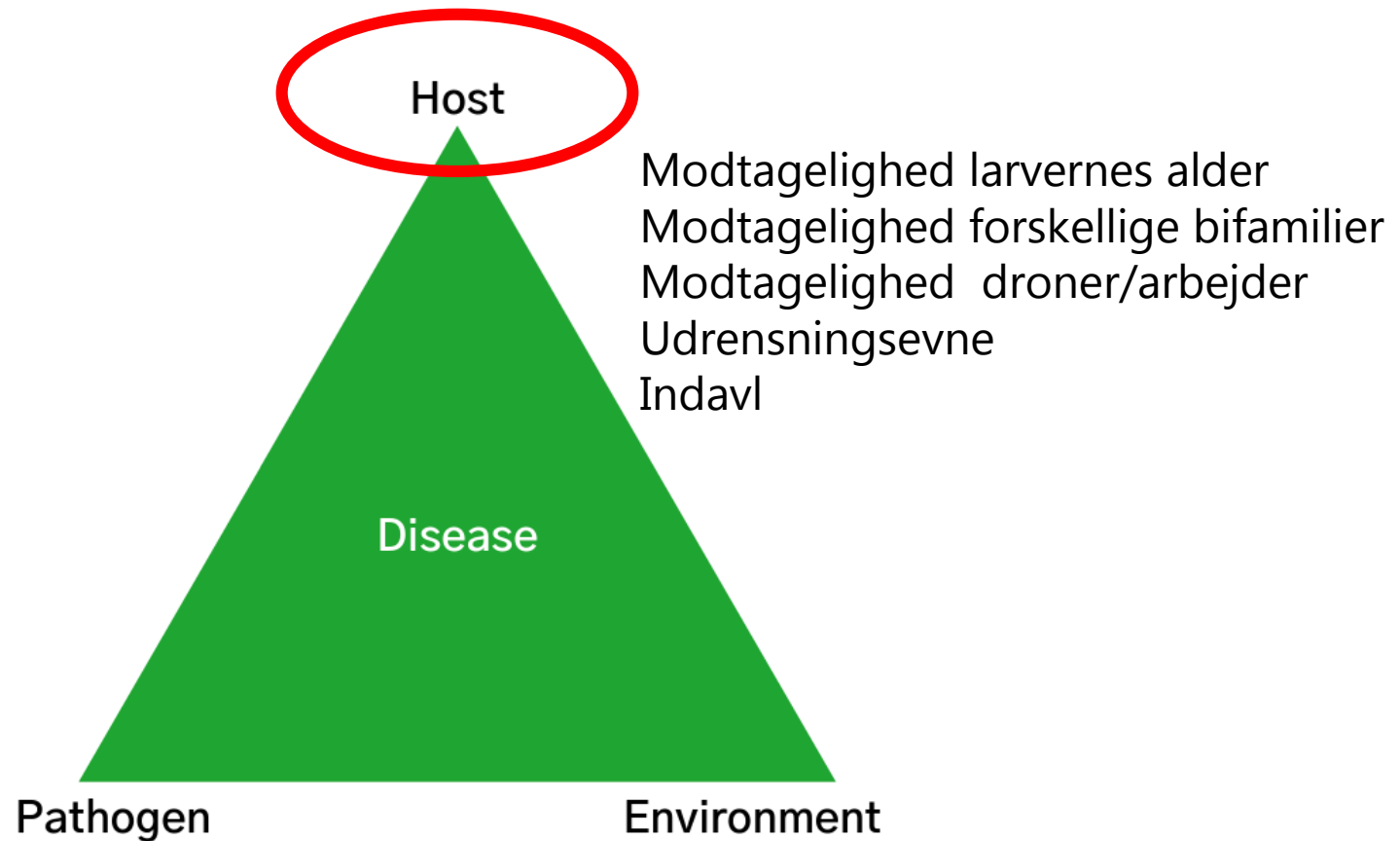
Genetic variation of *Ascospaera apis* and colony attributes do not explain chalkbrood disease outbreaks in Australian honey bees

Jody R. Gerdtz<sup>a</sup>, John M.K. Roberts<sup>b</sup>, Michael Simone-Finstrom<sup>c</sup>, Steven M. Ogbourne<sup>d</sup>, Joseph Tucci<sup>a</sup>

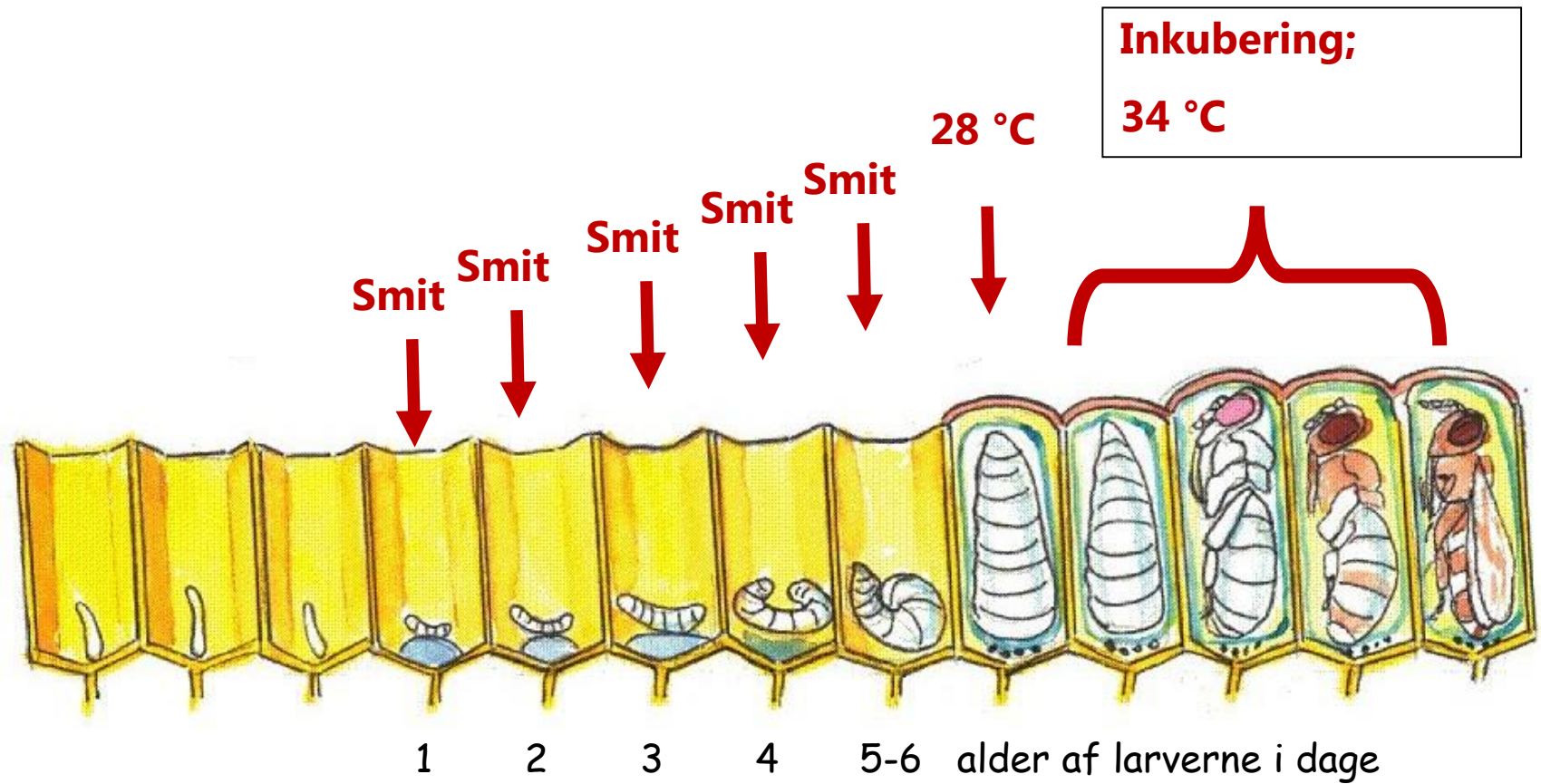


Mange bifamilier bliver inficeret af flere forskellige type kalkyngel – selv enkelte larver.

# Samspil mellem vært, pathogen og miljø



# Modtagelighed af bilaver i forhold til alder

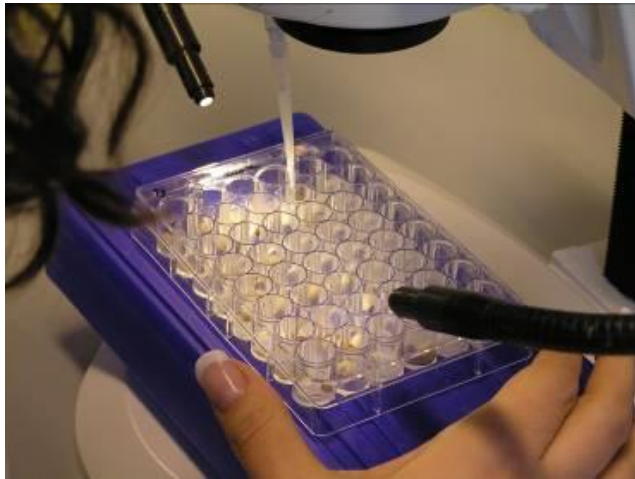


## Modtagelighed af bilaver i forhold til alder

2 x 48 larvae blev omlarvet seks dage i træ

På dag 6 fik larverne fra hver dag 5 µl foder med 500 *A. apis* spore.

En bifamilie, tre gentagelser



$$5 + 5 = 10 \text{ } \mu\text{l (day 1)}$$

$$5 + 5 = 10 \text{ } \mu\text{l (day 2)}$$

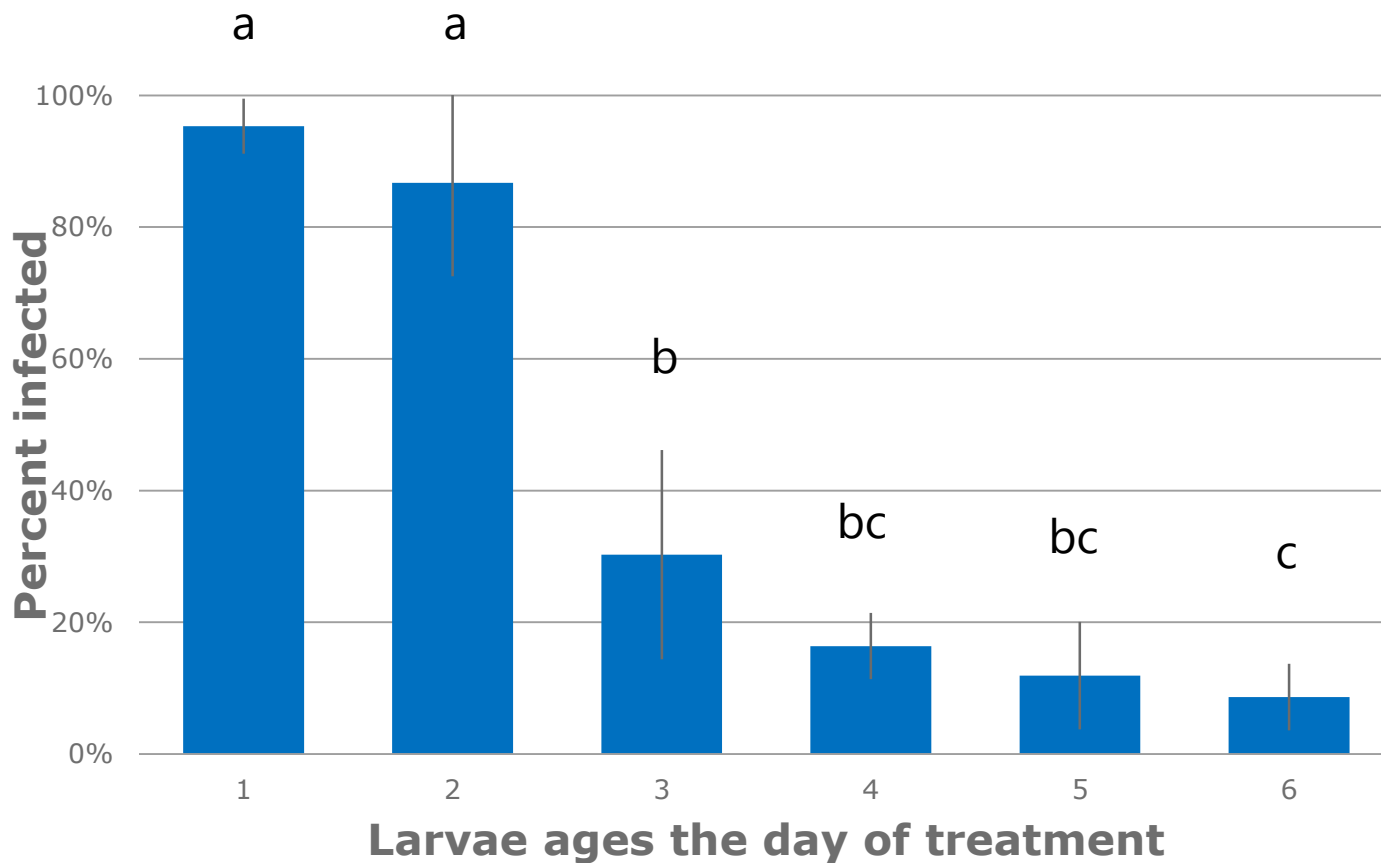
$$5 + 15 = 20 \text{ } \mu\text{l (day 3)}$$

$$5 + 25 = 30 \text{ } \mu\text{l (day 4)}$$

$$5 + 35 = 40 \text{ } \mu\text{l (day 5)}$$

$$5 + 45 = 50 \text{ } \mu\text{l (day 6)}$$

## De mindste larver er mest modtagelige



# Modtagelighed for forskellige underarter

Bifamilier med øparrede dronninger

4 familier *A. m. mellifera*

3 familier *A. m. ligustica*

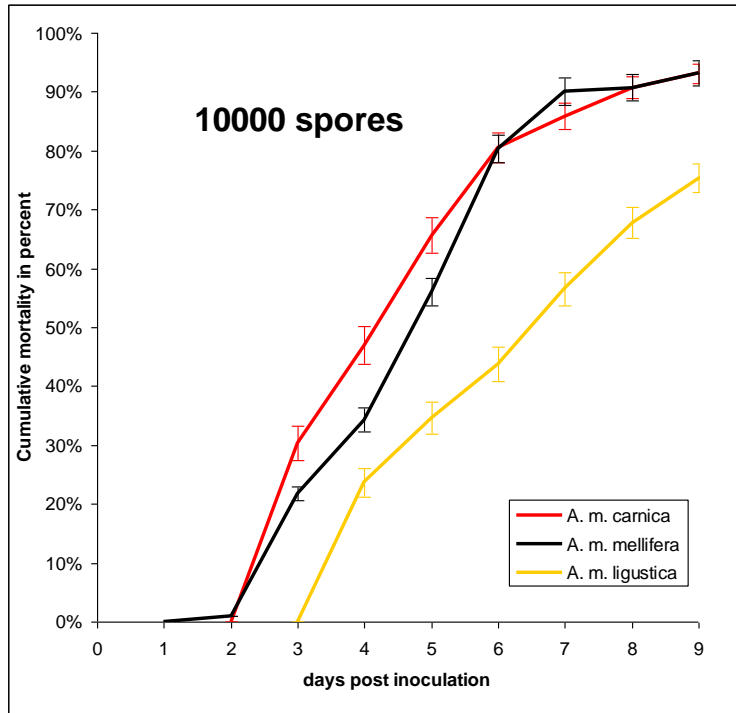
3 familier *A. m. carnica*

4 familier Buckfast (hybrid)





# Modtagelighed for forskellige underarter

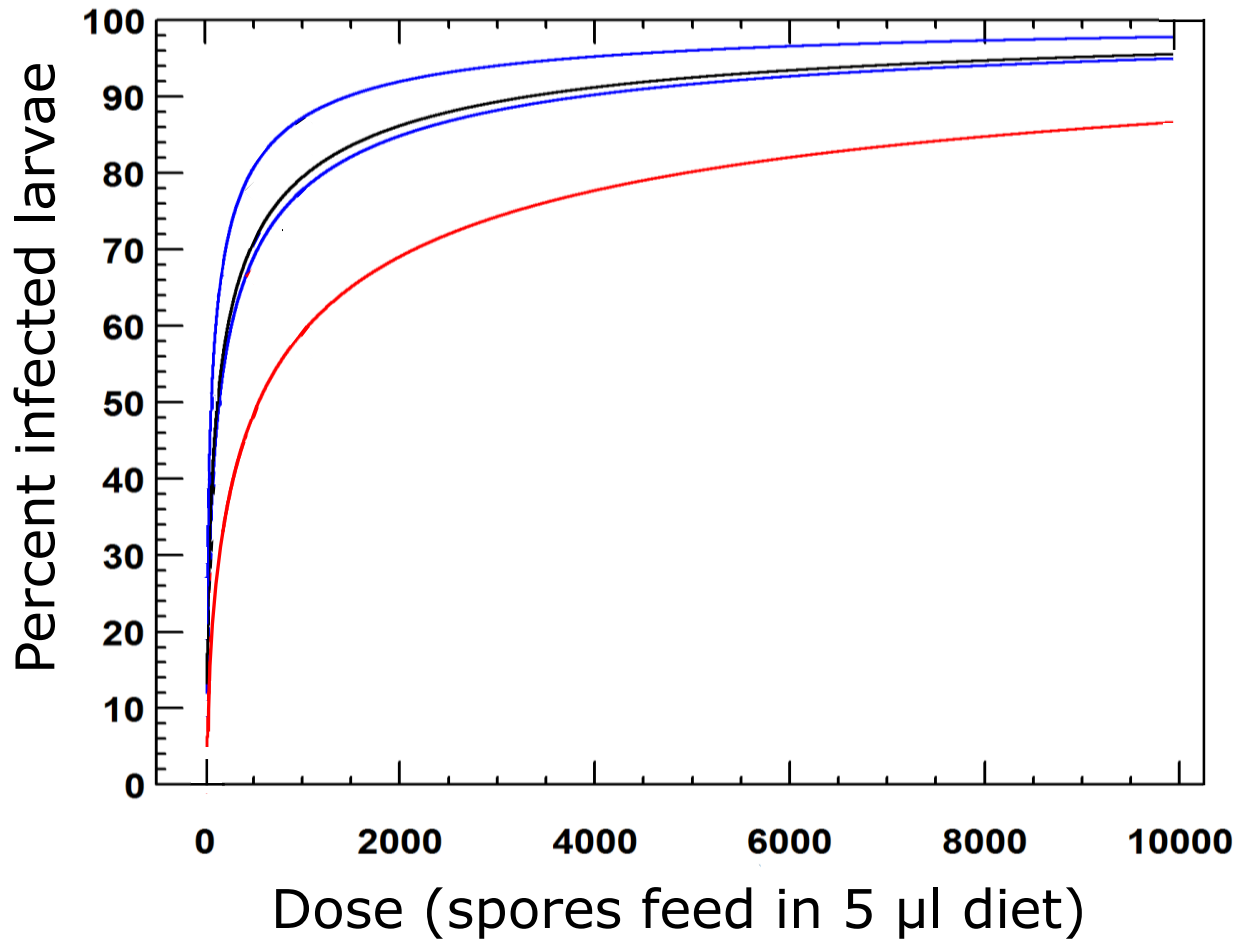


Subspecies	LD <sub>50</sub> (spores ingested)	Susceptibility
<i>A. m. mellifera</i>	70-300	High
<i>A. m. carnica</i>	50-500	Middel
<i>A. m. ligustica</i>	400-900	Low
Buckfast	350-1000	Low

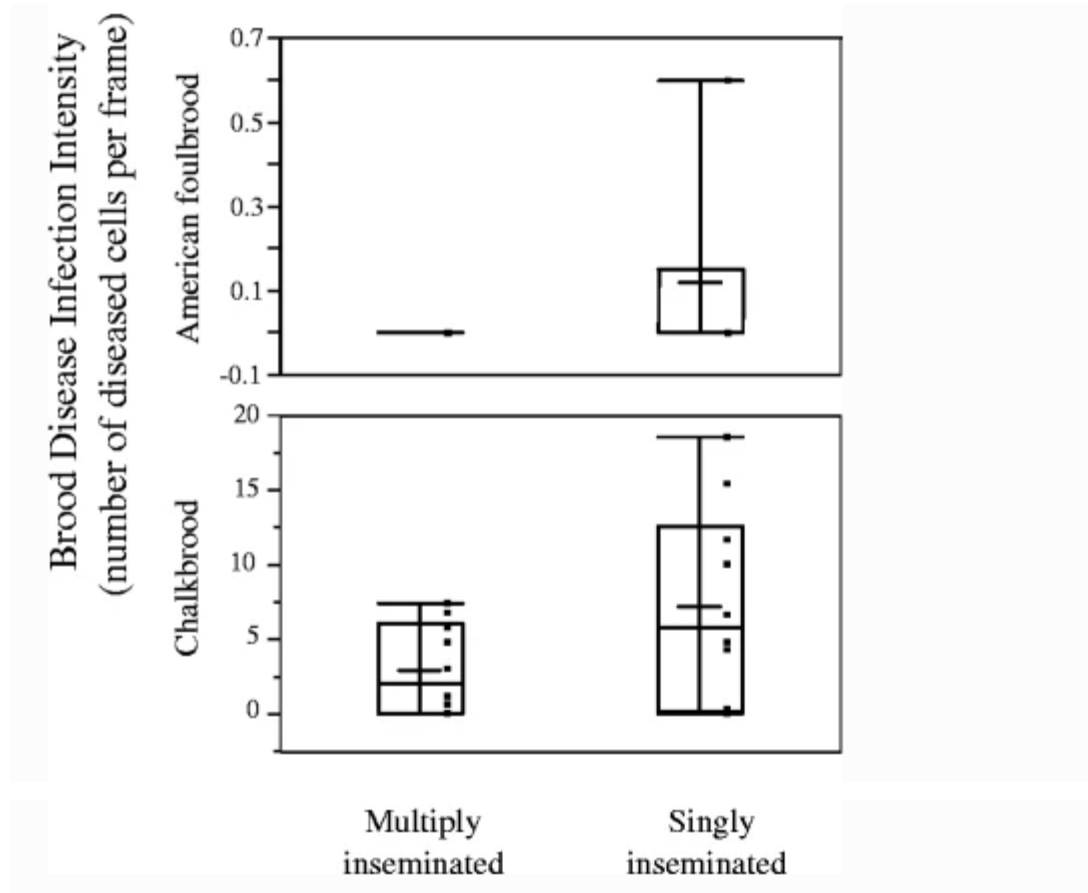


***carnica*    *mellifera*    *ligustica***

# Arbejderlarvernes modtagelighed varierer mellem bifamilierne inden for en underart



# Lav genetisk variation i bifamilier øger chancen for kalkyngel



## Samspillet mellem bierne og kalkyngel - 1

- Mindre larver er mere modtagelige
- Nogle bifamilier har større modstandskraft, der er stor variation selv inden for en underart
- Dronninger parret med flere droner klare sig bedst
- Dronelarver er mere modtagelige end arbejderlarver

# Social immunitet - Udrensning



- Arbejderbierne detekterer, åbner cellelågene og fjerner død og sygt yngel

# Social immunitet - Udrensning

## HYGIENIC BEHAVIOR OF HONEY BEES IN RELATION TO CHALKBROOD DISEASE

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U.S. Department of Agriculture, Agricultural Research Service  
Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, Arizona 85719  
and

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U.S. Department of Agriculture, Agricultural Research Service  
Colorado State University, Economics Building, Fort Collins, Colorado 80521

<https://doi.org/10.22319/rmcp.v13i1.5907>

Article

### Effect of hygienic behavior on resistance to chalkbrood disease (*Ascosphaera apis*) in Africanized bee colonies (*Apis mellifera*)

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Luis Abdelmir Medina Medina <sup>b</sup>

Ernesto Guzmán-Novoa <sup>c</sup>

<sup>a</sup> Universidad Autónoma de Zacatecas. Unidad Académica de Medicina Veterinaria y Zootecnia, Zacatecas, México.

<sup>b</sup> Universidad Autónoma de Yucatán. Departamento de Apicultura, Campus de Ciencias Biológicas y Agropecuarias. Carretera Mérida-Xmatkuil Km. 15.5, Mérida, Yucatán, México.

<sup>c</sup>School of Environmental Sciences, University of Guelph, Guelph, Canada.

Bifamilierne blev smittet med kalkyngel.

Alle fire artikler viser at der er en sammenhæng mellem udrensning af nåle/fryse dræbt yngel og kalkyngel.

## Honey Bee (Hymenoptera: Apidae) Hygienic Behavior and Resistance to Chalkbrood<sup>1</sup>

CHARLES P. MILNE, JR.

Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Ann. Entomol. Soc. Am. 76: 384-387 (1983)

**ABSTRACT** Honey bee, *Apis mellifera* L., hygienic behavior is the uncapping and removing of brood killed by American foulbrood. The magnitude of removing behavior was significantly correlated with colony resistance to chalkbrood, *Ascosphaera apis*, after infection by feeding spores to the colonies. Uncapping behavior, however, was not significantly correlated with colony resistance. Hygienic behavior confers resistance to both a bacterial and a fungal brood disease. Colonies at the same apiary had different susceptibilities to infection. This suggests that resistance to chalkbrood is complex and involves other mechanisms besides hygienic behavior.

## Neotropical Entomology

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### ECOLOGY, BEHAVIOR AND BIONOMICS

#### Resistance to Chalkbrood Disease in *Apis mellifera* L. (Hymenoptera: Apidae) Colonies with Different Hygienic Behaviour

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#### Keywords

Honey bee, *Ascosphaera apis*, larva, selection

#### Correspondence

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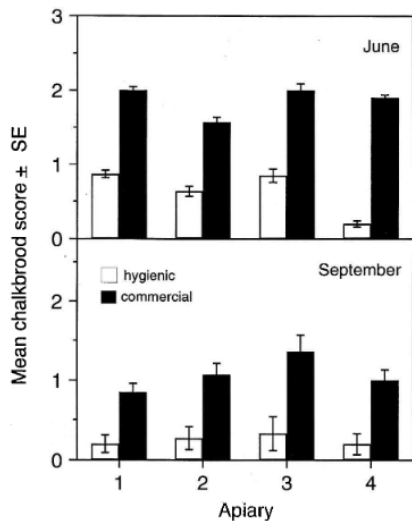
Edited by Kleber Del Claro - UFU

Received 03 August 2009 and accepted 10 June 2010

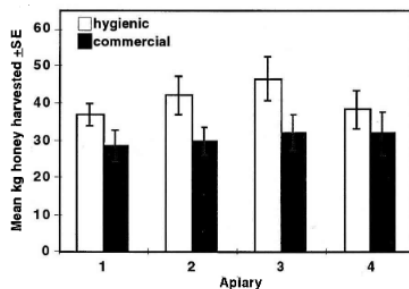
#### Abstract

Chalkbrood disease affects the larvae of honeybees *Apis mellifera* L. and is caused by the fungus *Ascosphaera apis*. Infected larvae die when they are stretched in the cap cell and suffer a gradual hardening that ends in a very hard structure (mummie). Several studies have demonstrated that colonies that express an efficient hygienic behaviour (uncapping of cell and subsequent removal of dead brood) exhibit a higher resistance to the disease. However, it remains unclear whether the advantage of hygienic colonies over less hygienic ones lies in the ability to remove mummies or in the early detection of infected larvae and its cannibalization before they harden. To elucidate this aspect, the hygienic behaviour of 24 colonies, which were subsequently provided with pollen cakes containing *A. apis*, was evaluated. The number of mummies and the number of partially cannibalized and whole larvae in uncapped cells were recorded. The most hygienic colonies controlled the disease better. These colonies also had a higher tendency to uncap cells that contained infected larvae and cannibalize them. The presence of *A. apis* in partially cannibalized and whole larvae in uncapped cells indicate that the advantage of hygienic colonies over less hygienic ones lies in the early detection of infected larvae death and their quick removal from the cell before they become mummies.

# Social immunitet - Udrensning



**Figure 2.** Abundance of chalkbrood mummies on two frames of capped brood within 49 hygienic and 46 commercial colonies distributed among four apiaries in June and September 1996. Colonies were scored from 0 to 3 (uninfected to highly infected), where 0 = no mummies, 1 = 1–5 mummies, 2 = 5–20 mummies, and 3 = over 20 mummies. The hygienic colonies had significantly less chalkbrood than the commercial colonies in both months ( $P < 0.001$ ).



**Figure 3.** Honey production by 49 hygienic and 46 commercial colonies, distributed among four apiaries, in early September 1996. The hygienic colonies produced significantly more honey than the commercial colonies ( $P = 0.002$ ).

Her blev bifamilierne ikke smittet med kalkyngel



RESEARCH ARTICLE

## Hygienic behaviour selection via freeze-killed honey bee brood not associated with chalkbrood resistance in eastern Australia

Jody Gerdtz<sup>1\*</sup>, R. Laurie Dewar<sup>2\*</sup>, Michael Simone Finstrom<sup>3\*</sup>, Trevor Edwards<sup>4†</sup>, Michael Angove<sup>1‡</sup>

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### OPEN ACCESS

**Citation:** Gerdtz J, Dewar RL, Simone Finstrom M, Edwards T, Angove M (2018) Hygienic behaviour selection via freeze-killed honey bee brood not associated with chalkbrood resistance in eastern Australia. PLoS ONE 13(11): e0203969. <https://doi.org/10.1371/journal.pone.0203969>

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**Data Availability Statement:** All relevant data are within the manuscript and its Supporting

### Abstract

Hygienic behaviour is a social immune response in honey bees shown to help provide resistance to honey bee pests and diseases. A survey of hygienic behaviour and brood diseases was conducted on 649 colonies in eastern Australia to initiate a selective breeding program targeting disease resistance and provide a level of resistance to *Varroa* (*Varroa destructor* Anderson and Trueman and *V. jacobsoni* Oudemans) mites should they become established in Australia. The test population showed a remarkably high baseline level of hygienic behaviour with 17% of colonies meeting or exceeding breeding selection thresholds. Colonies belonging to a breeding program were 5.8 times more likely to be highly hygienic and colonies headed by queens raised from hygienic queen mothers were 2.2 times more likely. Nectar availability (nectar yielding flowering plants within honey bee forage range) influenced hygienic behaviour expression but was not a significant predictor of level of hygienic behaviour. Surprisingly, hygienic behaviour was not a significant predictor of the presence of infection of the honey bee brood disease chalkbrood (*Ascosphaera apis*) and was not influential in predicting severity of chalkbrood infection in surveyed honey bee colonies. This study, along with reports from commercial beekeepers that chalkbrood infection is on the rise, warrants a deeper exploration of the host-pathogen relationship between *Apis mellifera* and *Ascosphaera apis* in Australia.

Spivak and Reuter 1998, Apidologie

Hvornår kan udrensning være godt eller skidt i forhold til kalkyngel?

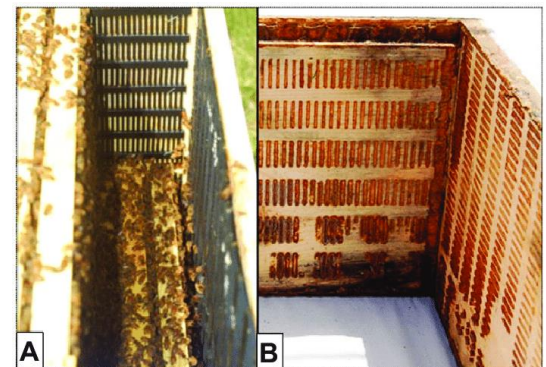
Sorte/hvide mumier!

# Social immunitet - Propolis

<b>Colony</b>	<b>Challenge</b>	<b>CB Mummies</b>	<b>CB Mummies</b>	<b>Total</b>	
<b>Treatment</b>		<b>Count 1</b>	<b>Count 2</b>		
Resin-poor	Unchallenged	0	0	0	<b>A</b>
	Chalkbrood	42.3±25.1	65.8±38.2	108.2±49.0	<b>B</b>
Resin-rich	Unchallenged	3.2±1.6	2.2±1.4	5.3±1.7	<b>A</b>
	Chalkbrood	1±0.5	13.7±7.2	14.7±7.5	<b>B</b>

Simone-Finstrom and Spivak, 2012 pOne.

Propolis hæmmer kalkyngel





# Tarmfloraen kan også have betydning



Article

## The *Ascospaera apis* Infection (Chalkbrood Disease) Alters the Gut Bacteriome Composition of the Honeybee

Dae Yoon Kim <sup>1,\*</sup>, Soohyun Maeng <sup>2,\*</sup>, Sung-Jin Cho <sup>3,\*</sup>, Hui Jin Park <sup>4</sup>, Kyungsu Kim <sup>3</sup>, Jae Kwon Lee <sup>4,\*</sup> and Sathiyaraj Srinivasan <sup>2,\*</sup>

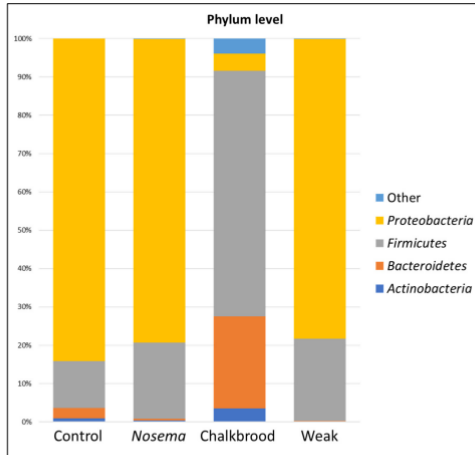


Figure 2. The phylum-level comparison between the *Nosema*, Chalkbrood, and weak honeybee gut bacterial communities.

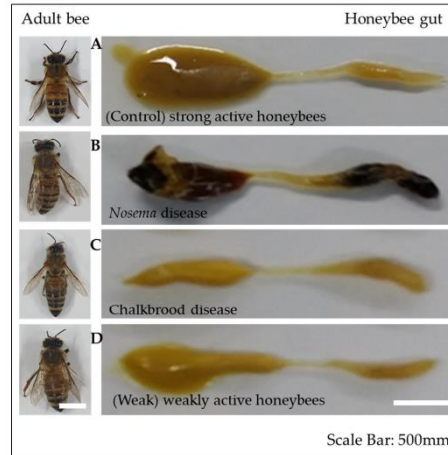


Figure 1. The dissection of the digestive tract of (A) control, (B) *Nosema*, (C) Chalkbrood, and (D) weak honeybees. The honeybees are dissected, and the gut contents were used for the analysis.

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### Strains of *Lactobacillus* spp. reduce chalkbrood in *Apis mellifera*

Marcos Raúl Tejerina <sup>a,b,\*</sup>, María José Cabana <sup>a</sup>, Marcelo Rafael Benitez-Ahrendts <sup>a,b</sup>

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M.R. Tejerina et al.

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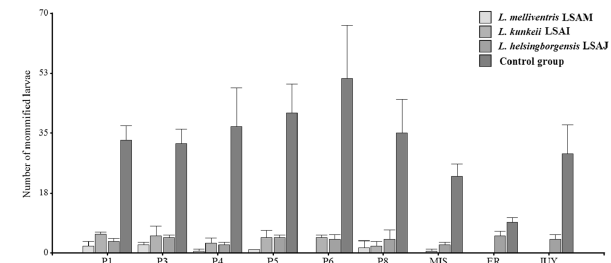


Figure 1. Number of mummified larvae that received three treatments of lactic bacteria.

# Modstandskraft over for kalkyngel og gener? – kromosom 11

Journal of Apicultural Research 51(2): 154-163 (2012)  
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ORIGINAL RESEARCH ARTICLE

## Association of single nucleotide polymorphisms to resistance to chalkbrood in *Apis mellifera*

Beth Holloway<sup>1\*</sup>, H Allen Sylvester<sup>1</sup>, Lelania Bourgeois<sup>1</sup> and Thomas E Rinderer<sup>1</sup>

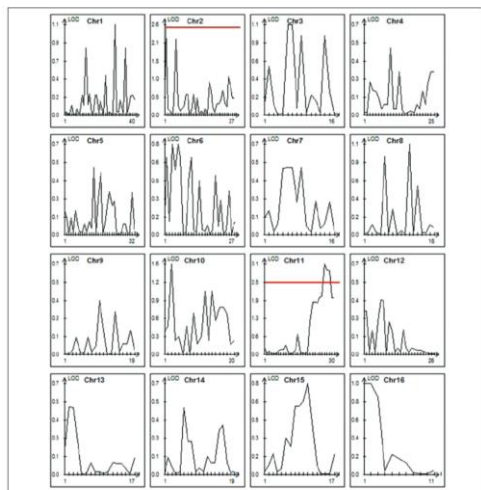


Fig. 1. SNP association peaks for chalkbrood resistance in honey bees. QTL Cartographer single marker analysis identifies associations between SNP and the chalkbrood resistance trait per chromosome. A LOD score threshold value of >2.5 (red line) shows that only associations on chromosomes 2 and 11 suggest a genetic basis for resistance. The x-axis for each graph shows the number of markers mapped to each.



Original Communication

## Validation of genetic markers associated with chalkbrood resistance

Katherine Aronstein<sup>a</sup>, Deanna Colby and Beth Holloway 2015

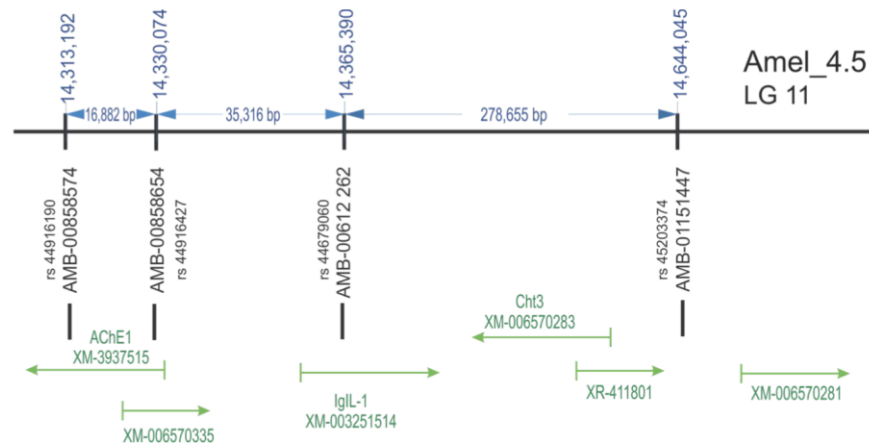


Figure 1. Amel\_4.5 LG 11 map showing genomic location of SNPs associated with chalkbrood resistance (rs44679060 and rs44916427) [6] and two additional SNPs flanking this region (rs44916190 and rs45203374). Numbers (blue) above the line indicate location and distances between SNPs; NCBI accession numbers (black) are shown below the line. Arrows (green) below the line show location and transcription direction of the genes containing the SNPs, gene names (if known) and NCBI accession numbers.

A earlier series of SNPs on chromosome 11 found to related to chalkbrood resistance.



### Changes in the gene expression of chalkbrood resistance in *Apis mellifera* larvae infected by *Ascosphaera apis*

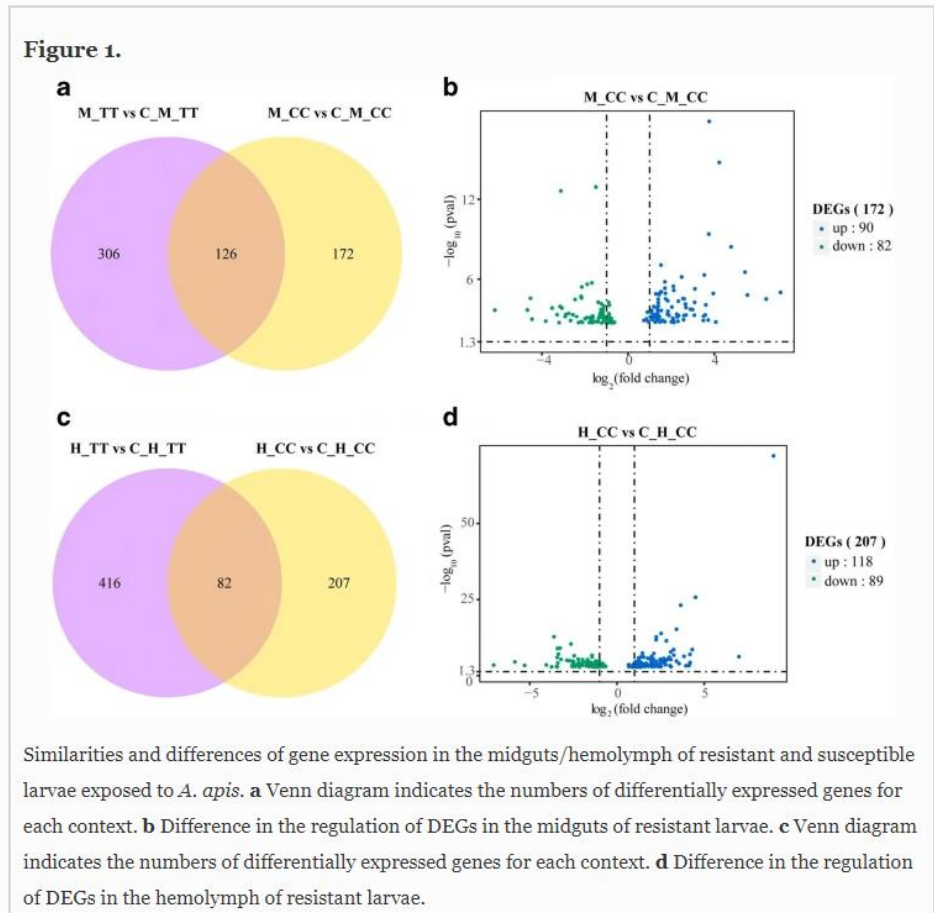
Hongyi NIE<sup>1</sup>, Xueyan WANG<sup>1</sup>, Shupeng XU<sup>1</sup>, Yan GAO<sup>1</sup>, Yan LIN<sup>1</sup>, Yanan ZHU<sup>1</sup>, Donglin YANG<sup>2</sup>, Zhiguo LI<sup>1</sup>, Songkun SU<sup>1</sup>

Bilarver fra tre raske familier blev inficeret med *A. apis* eller vand (kontol) i laboratoriet.

Efter 3 dage ekstraherede de DNA fra larverne.

De grupperede larverne som resistente eller modtagelige på basis af en SNP på kromosom 11.

172 and 207 gener blev udtrykt forskelligt i tarm og hæmolymfen hos de laver der var grupperede som resistente.



Apidologie (2020) 51:35–47  
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 DOI: 10.1007/s13592-019-00702-y

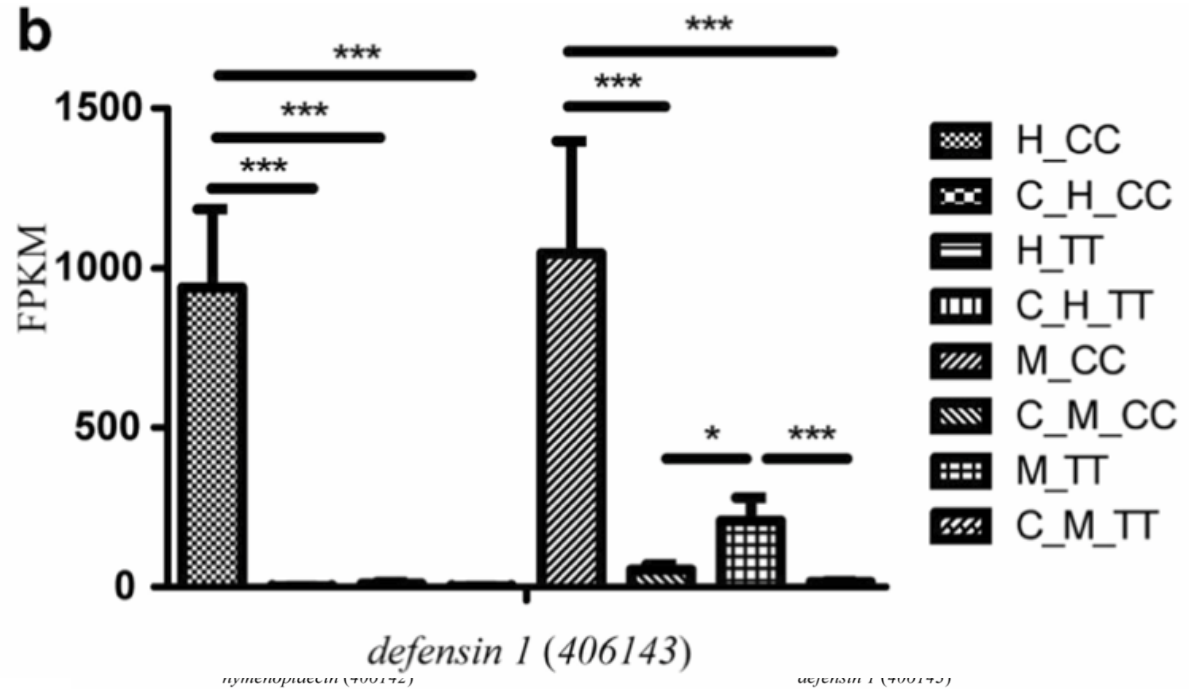


**Changes in the gene expression of chalkbrood resistance in *Apis mellifera* larvae infected by *Ascosphaera apis***

Hongyi NIE<sup>1</sup>, Xueyan WANG<sup>1</sup>, Shupeng XU<sup>1</sup>, Yan GAO<sup>1</sup>, Yan LIN<sup>1</sup>, Yanan ZHU<sup>1</sup>, Donglin YANG<sup>2</sup>, Zhiguo LI<sup>1</sup>, Songkun SU<sup>1</sup>

Immun response blev undersøgt yderligere.

Det antimicrobiel peptide defensin 1 blev opreguleret i hæmolymfen hos de bier de var smittet og typet som resistente (CC)



**Figure 3.** *Hymenoptaecin* and *defensin 1* gene expressions by FPKM and qRT-PCR. **a** and **b** The FPKM values of expression profiles in the midguts and hemolymph of resistant/susceptible larvae exposed to *A. apis*, respectively. **c** and **d** The qRT-PCR values of expression profiles in the midguts and hemolymph of resistant/susceptible larvae exposed to *A. apis*, respectively. H\_CC represents the hemolymph of the C/C larvae from the treatment group; C\_H\_CC represents the hemolymph of the C/C larvae from the control group; H\_TT: represents the hemolymph of the T/T larvae from the treatment group; C\_H\_TT: represents the hemolymph of the T/T larvae from the control group; M\_CC: represents the midguts of the C/C larvae from the treatment group; C\_M\_CC: represents the midguts of the C/C larvae from the control group; M\_TT: represents the midguts of the T/T larvae from the treatment group; C\_M\_TT: represents the midguts of the T/T larvae from the control group.

## Kalkyngel prøver

Yngelceller blev åbnet og to næsten færdig bier og to mumier blev testet på SmartBees SNP panel.

36-40 % SNP virkede ikke..

DNA kvaliteten I mumierne var for dårlig!



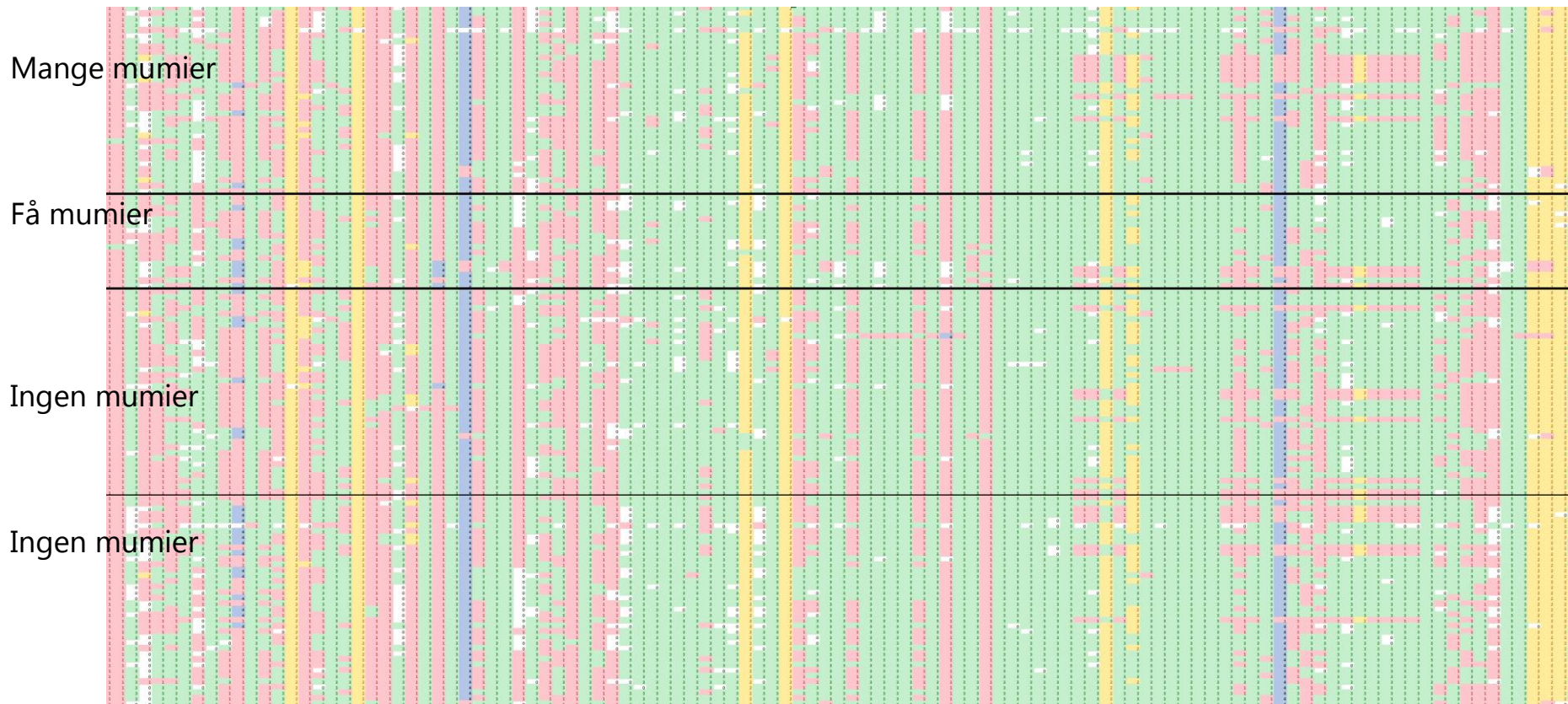
# Samling af syge og raske bifamilier

Isabell modtog og indsamlede prøver fra syge og raske bifamilier i same bigård.

Vi vidste altså at kalkyngel var tilstede og vi kunne finde sygdomstrykket i pøverne.



# Vi kunne kun undersøge raske bier







# Significant?

Numrene var  
signifikante

Men bierne var også  
beslægtede – hvorfor  
de delte alleler.

SNP1	Many mummies	Few mummies	No mummies
AA	8	1	0
AC	14	8	14
CC	12	8	18
SNP1	Many mummies	Few mummies	No mummies
AA	13	12	27
AG	13	5	7
GG	8	0	0

## Vi arbejder videre

- Undersøge flere bier
- Vi er ved at udvikle nye SNPs
- Hvis der er noget med de SNPs på kromosom 11 så er kan det bruges til at avle for resistens mod kalkyngel



# Hvad gør I?

Linjer med kalkyngel selekteres fra (genetikken)

Undgå indavl?

Sikre udrensningsevne?