

Breeding for Disease and Mite Resistance



Marla Spivak
University of Minnesota
www.beelab.umn.edu

New Breeding Program

Selecting for Health





Social Immunity

How social insects maintain the health of the colony
(Cremer et al., 2007)



Care - Kill Strategies of Social Immunity

Care - Prevention

- Grooming
- Antimicrobial secretions
- Nest sanitation

Kill – Once infected

- Sacrifice infectious individuals



Sylvia Cremer
Ants

Tragust S, Mitteregger B, Barone V, Konrad M, Ugelvig LV, and Cremer S

Ants Disinfect Fungus-Exposed Brood by Oral Uptake and Spread of Their Poison

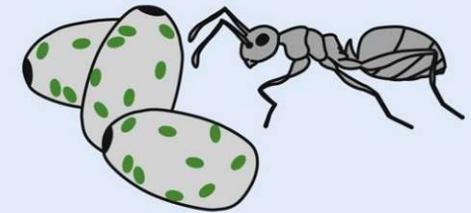


Lasius neglectus

Care

1. Groom fungal pathogen, *Metarhizium*, off brood
2. Disinfect with venom

Ants tending pathogen-exposed brood



Antimicrobial activity of poison substances



Kill

Destructive disinfection of infected brood prevents systemic disease spread in ant colonies

Ants target pupae infected with *Metarhizium* **when pathogen in non-contagious incubation period**, using chemical 'sickness cues' emitted by pupae

Ants remove the pupal cocoon, dismember pupa, and inject antimicrobial venom



Pull et al., 2018 eLife

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Care and Kill strategies for disease *and* mite resistance



.... A walk through my thought process of how honey bees use Care and Kill strategies, and how we can use this information to guide breeding programs

Honey bee hygienic behavior is a Kill strategy



Photo: K. Wagoner

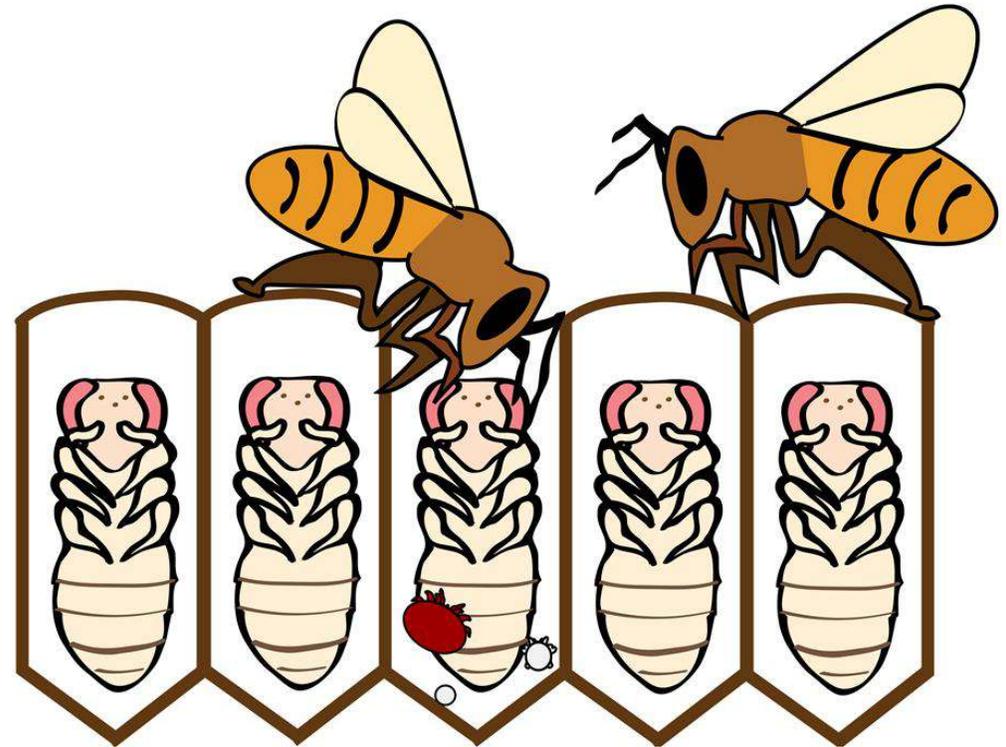


Photo: A. McKafee

Hygienic behavior is a Kill strategy with critical detection and timing components

Woodrow and Holst (1942) “...resistance to American foulbrood in the honey bee colony consists in its ability to ***detect and remove diseased brood before the causative organism... reaches the infectious spore stage*** in the diseased larvae.”



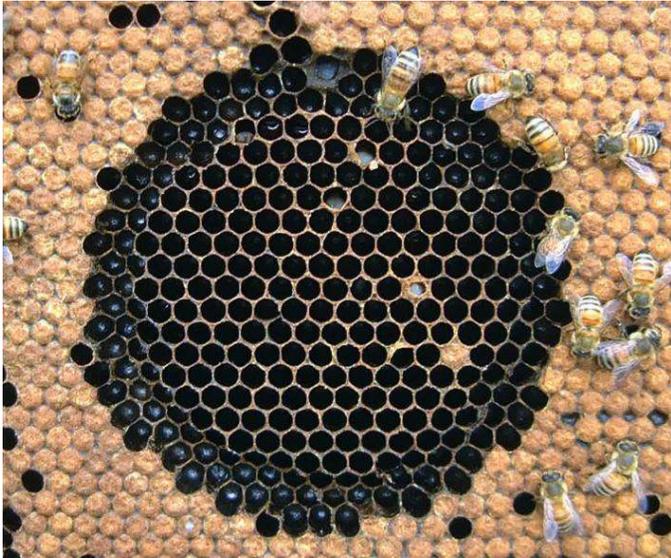
How do honey bees detect diseased brood?

Do honey bees have a *chemical recognition template* of “healthy brood” and thus detect “abnormal” brood of any kind (like immune system and nestmate recognition)?



“Minnesota Hygienic Line”

1. Assay freeze-killed brood



>95% removed in 24h
Breeders

2. Propagate



3. Challenge with pathogen or parasite



Mechanisms underlying Hygienic Behavior



ODOR

Hygienic behavior mediated by olfactory cues from diseased brood

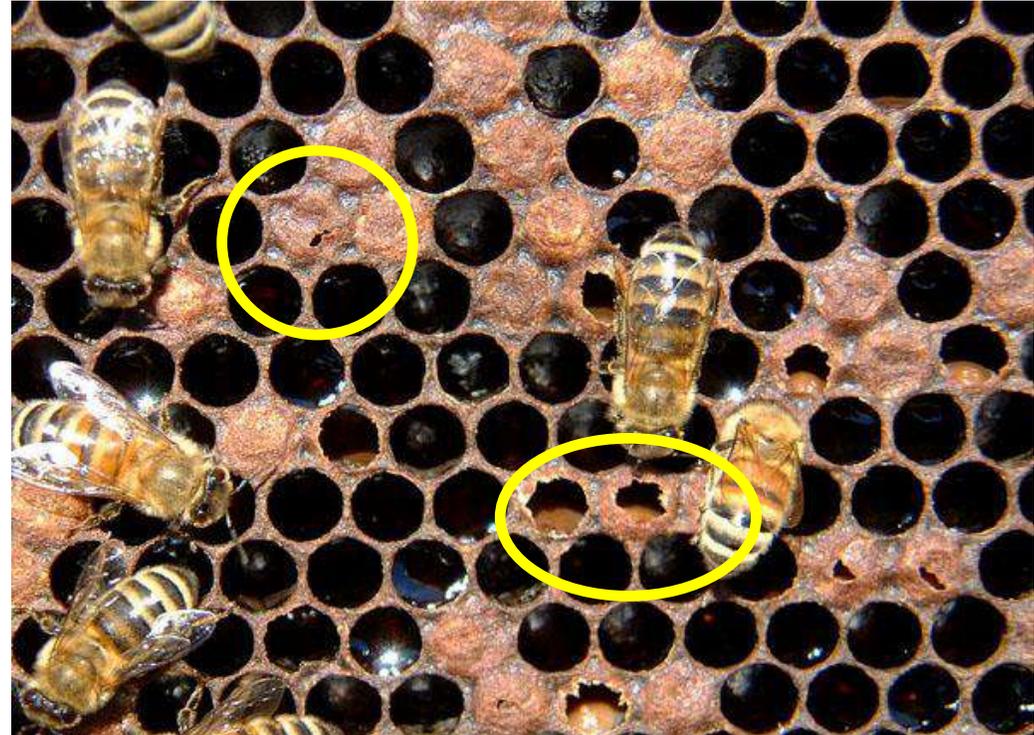
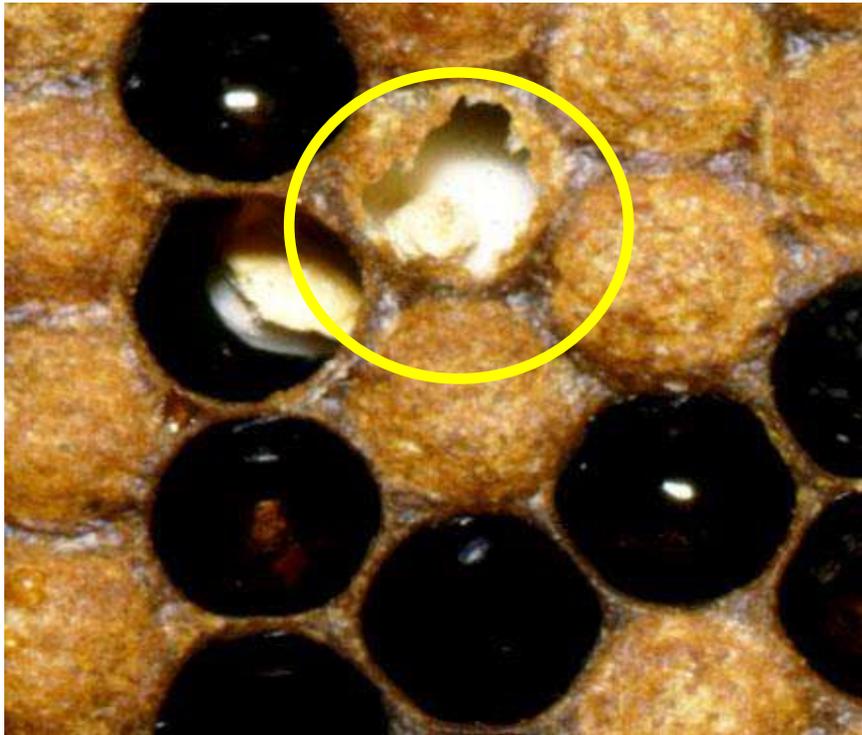


RESPONSE THRESHOLD

Hygienic bees are able to detect and discriminate between odors of diseased and healthy brood at low stimulus level

Arathi et al., 2001; Arathi & Spivak, 2006; Gramacho & Spivak, 2003; Masterman et al., 2001; Spivak et al., 2003; Swanson et al., 2009.

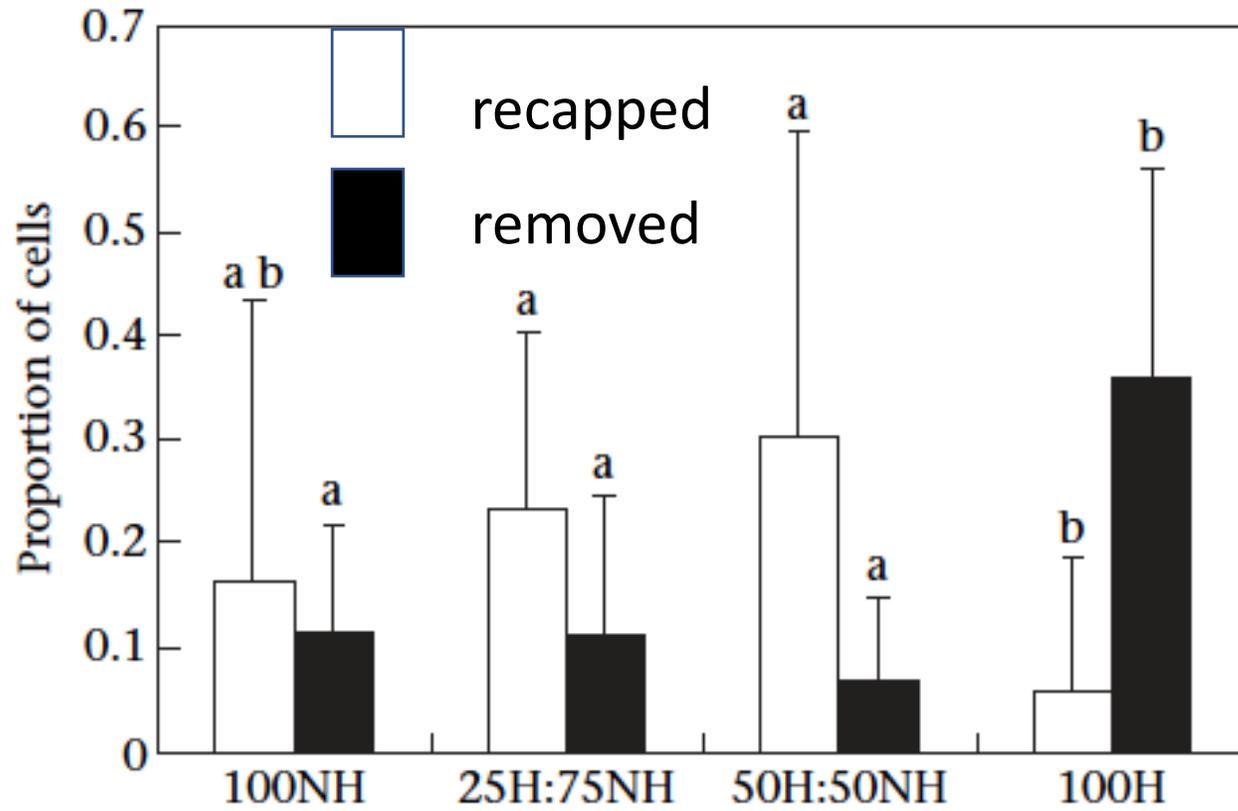
Non-hygienic bees do detect and remove diseased brood,
but when pathogen is infectious
And when stimulus level potentially is very high



Arathi et al., 2001
Arathi & Spivak, 2006

Why all the Uncapping and Recapping?

...depends on proportion of hygienic bees in colony

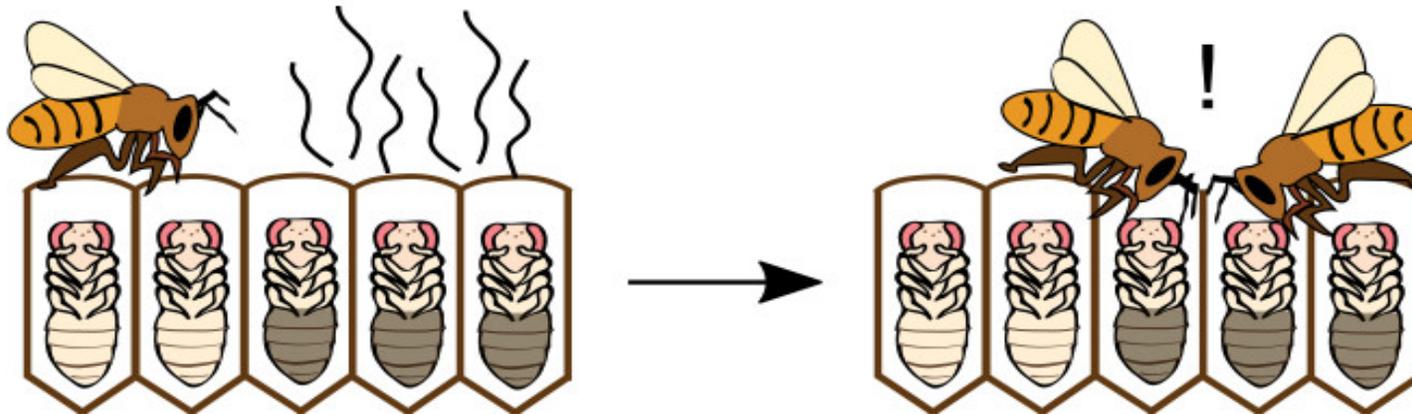


We thought: Uncapping- Recapping was “...inefficient task partitioning [that] would allow the pathogen to reach the infectious stage and increase the probability of disease transmission”

Arathi and Spivak, 2006. *Animal Behav*
Spivak and Gilliam, 1993. *J. Apic. Res*

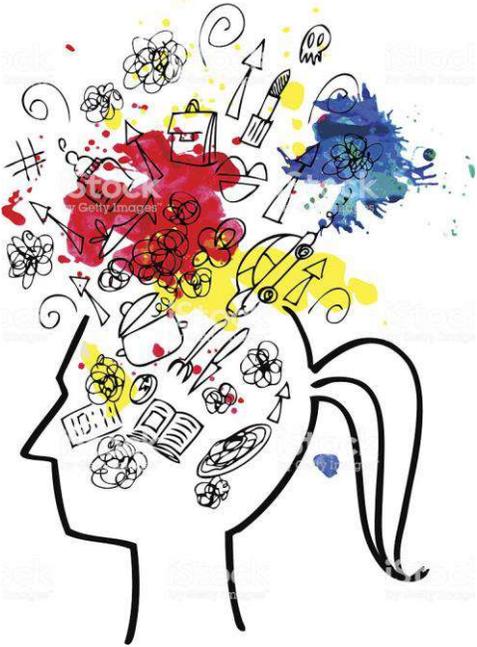
Critical timing to detect and remove diseased brood

- Pathogen advantage: detectable when infectious
- Colony advantage (altruistic): detectable before infectious



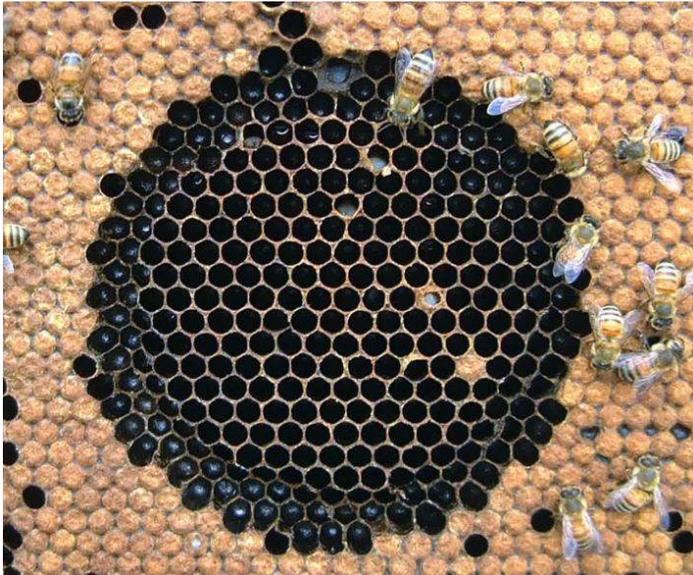
Varroa destructor

What is relationship between hygienic removal of diseased brood vs mite infested brood?



Can bees selected based on freeze-killed brood assay (Minnesota Hygienic Line) detect and remove mite-infested brood?

Assay freeze-killed brood

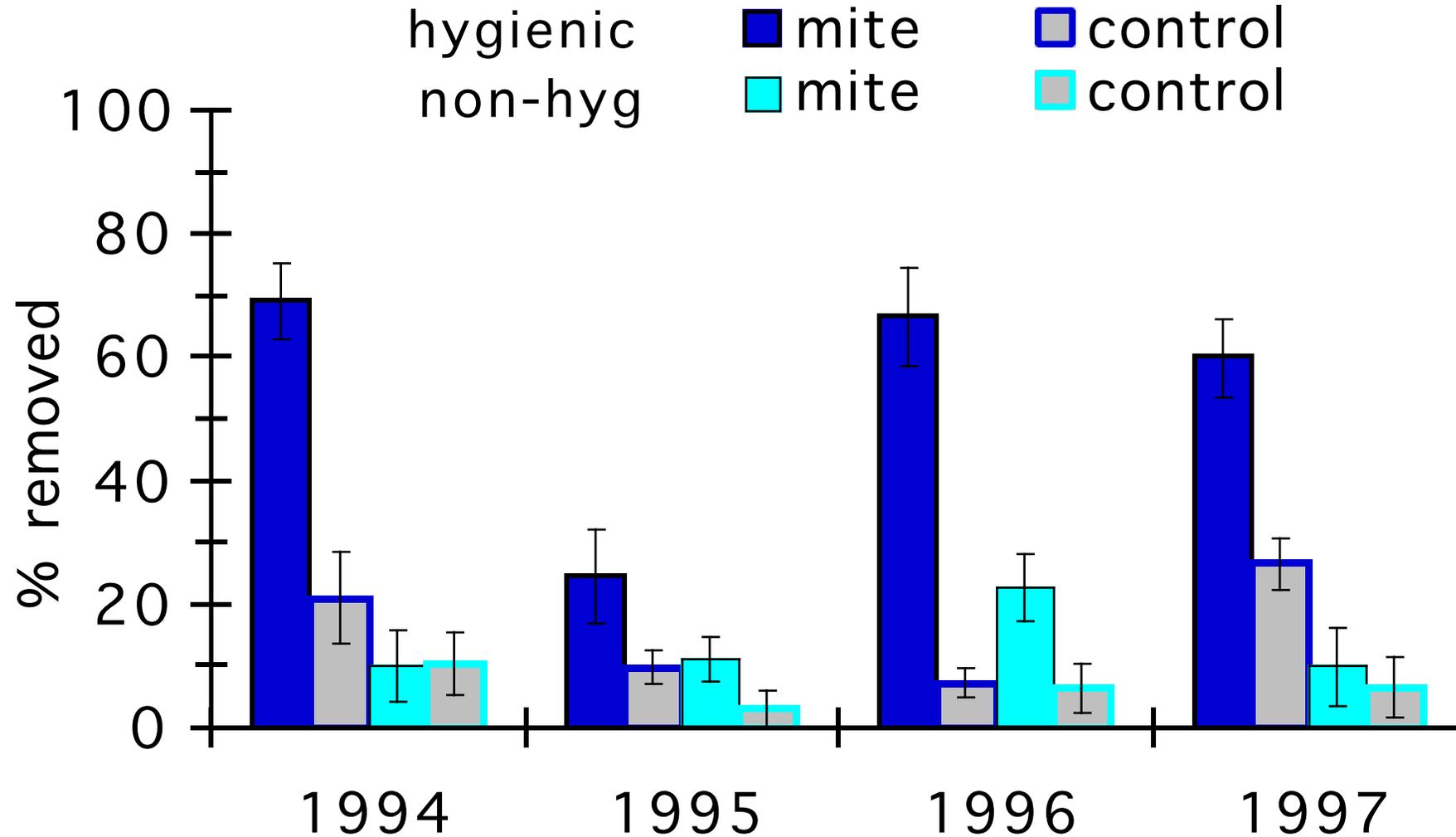


Challenge with *Varroa*



- Introduced mites into cells 4-6 hrs after cell capping
- Recorded removal of mite-infested pupae daily

Removal of mite-infested brood



Spivak, 1996 *Apidologie*; Spivak and Gilliam, 1998

Suppression of Mite Reproduction line = SMR

Low mite reproductive success (Harbo and Hoopingarner, 1997)

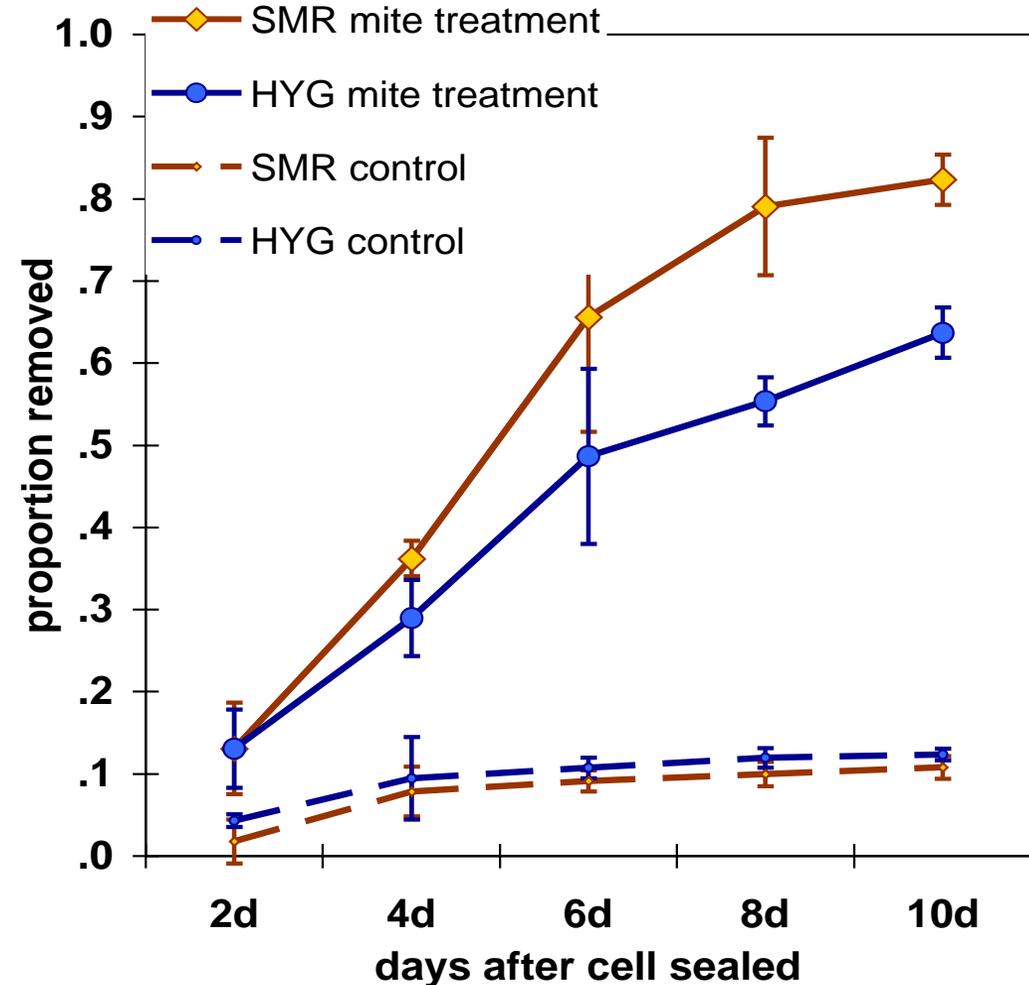


Idtools.org

Harbo & Harris
USDA-ARS
Baton Rouge

Difference between SMR and MN Hygienic line?

- SMR remove higher % mite-infested SMR brood (more hygienic)
- Mites in colonies have lower reproductive success, especially on own brood



Ibrahim et al. 2006

Harbo and Harris 2005

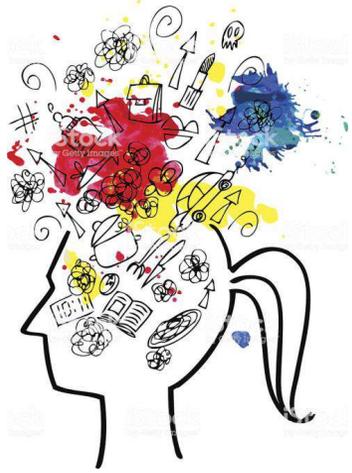
Suppression of Mite Reproduction line = SMR

RENAMED

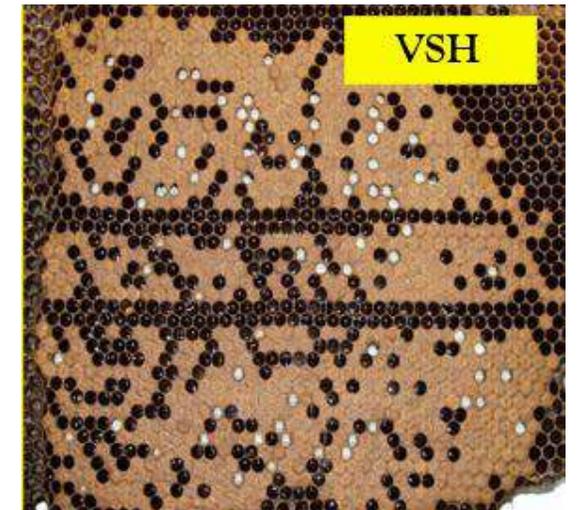
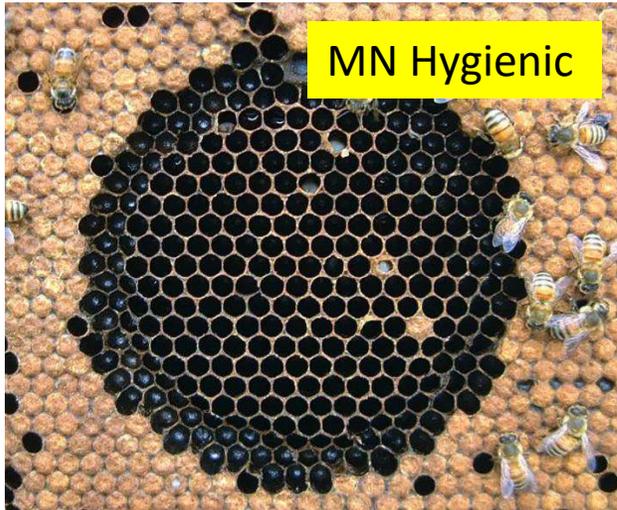
Varroa Sensitive Hygiene line = VSH

Harbo & Harris, 2005

Are “Hygienic” and “VSH -Varroa Sensitive Hygiene” different behavioral traits?



Can any colonies bred for VSH behavior detect and remove diseased brood, before pathogen forms infectious spores?



Care - Kill Strategies of Social Immunity

“Selection for detection of illnesses by healthy adults and the ‘advertisement’ of disease status by sick individuals”

S. Cremer



Can we identify brood
“advertisement” and use it in
breeding programs as a *Varroa*-
specific assay?

SCIENTIFIC REPORTS



OPEN

Specific Cues Associated With Honey Bee Social Defence against *Varroa destructor* Infested Brood

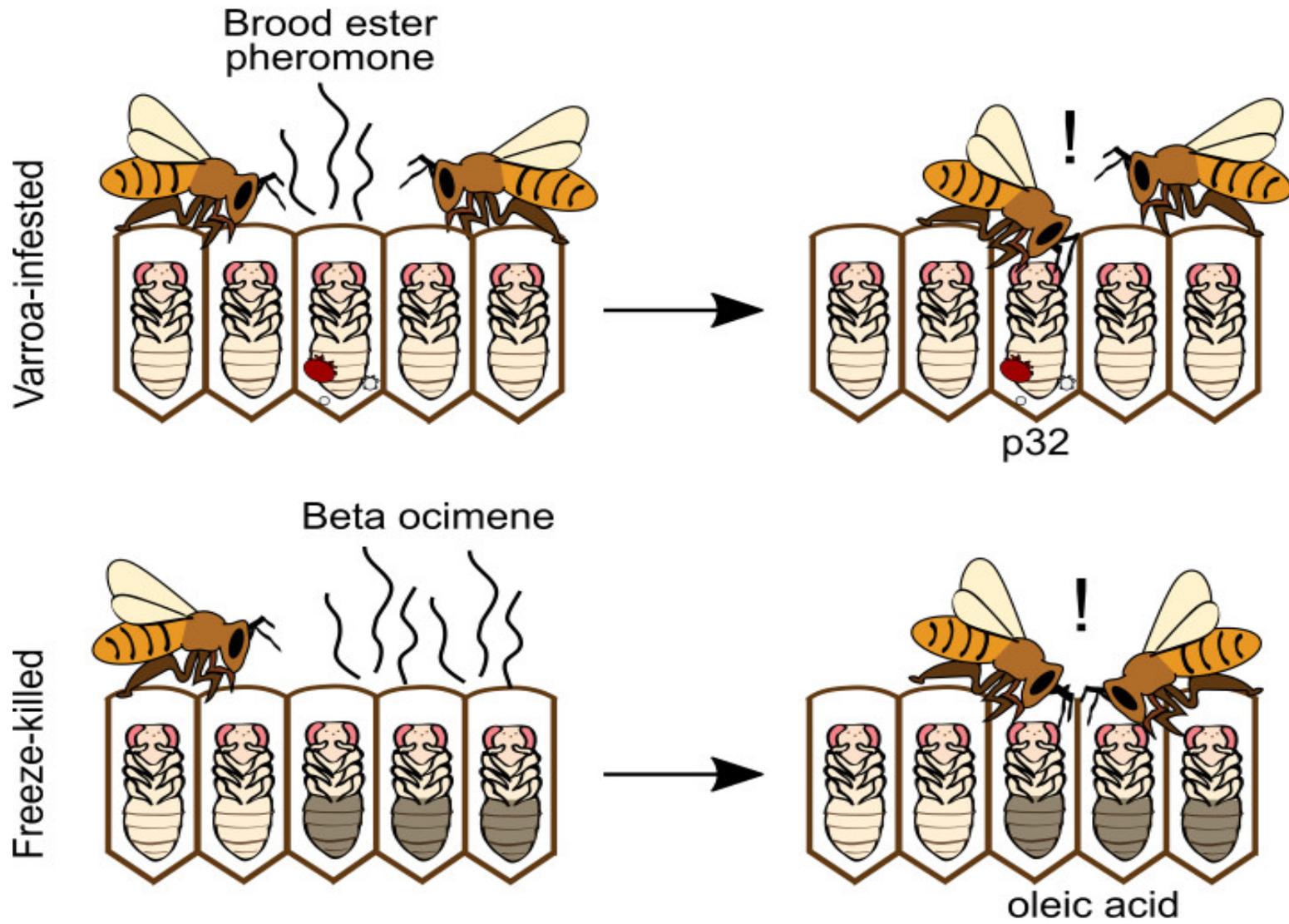
Received: 06 January 2016

Accepted: 15 April 2016

Published: 03 May 2016

Fanny Mondet^{1,2}, Seo Hyun Kim², Joachim R. de Miranda³, Dominique Beslay¹, Yves Le Conte¹
& Alison R. Mercer²

Mondet F, Kim S-H, de Miranda JR, Beslay D, Le Conte Y, Mercer AR (2016)



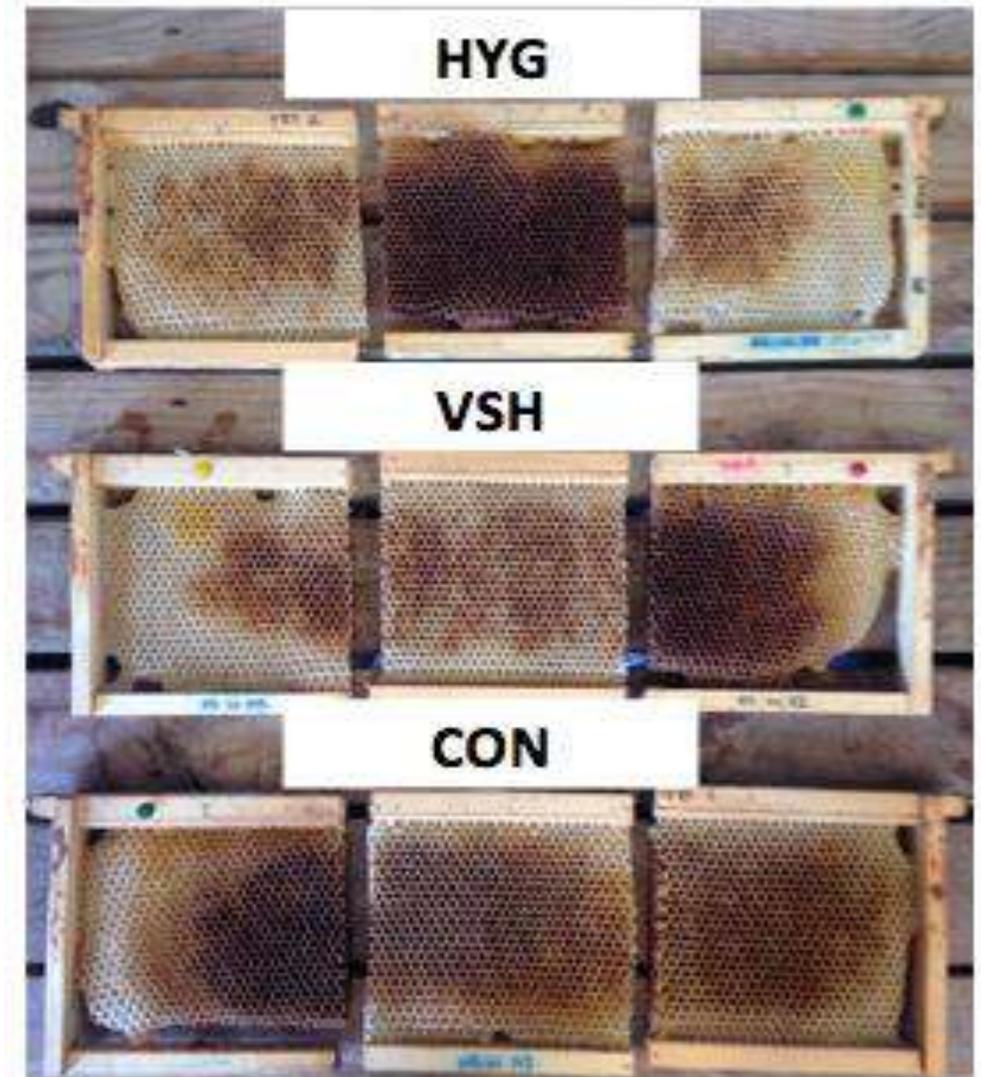
McAfee, A... Foster, L et al. 2017 bioRxiv

Stock-specific chemical brood signals are induced by *Varroa* and Deformed Wing Virus, and elicit hygienic response in the honey bee

K. Wagoner¹, M. Spivak², A. Hefetz³, T. Reams⁴ & O. Rueppell¹

Wagoner, Spivak, Ruepell. 2018. *J. Econ. Ent.*

Wagoner et al. 2019. *Sci Reports*



Cross-fostering experiments and chemical analyses



Use of *Varroa*-specific brood
“advertisement” as an assay in
breeding programs would be great

....but will it also help select for
disease *and virus* resistance?



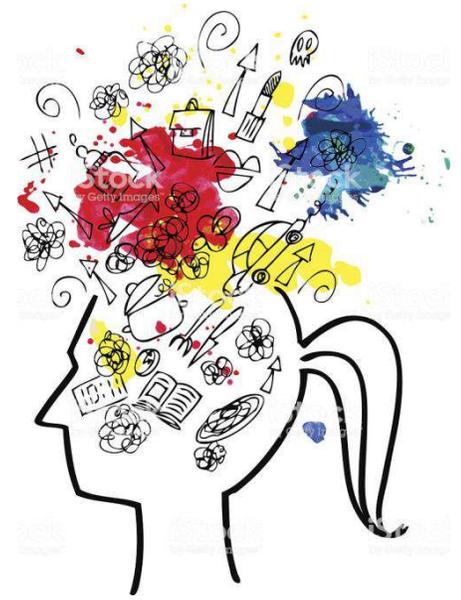
Is there a critical timing for detection and removal of mite infested and virus infected brood?

Detection and removal begins when mite starts oviposition

Boecking, 1992

Spivak, 1996

Harris, 2007

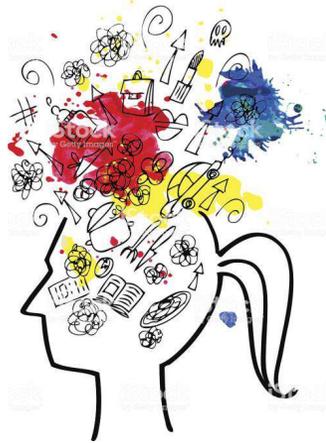


Is uncapping and recapping behavior good or bad?

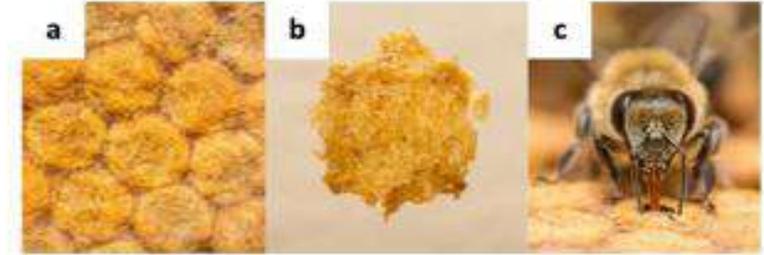
Active defense against *Varroa*?

or

Inefficiency, allowing pathogen to reach infectious stage?



Harris et al. 2010. *Annals ESA*
Oddie et al., 2018. *Sci. Reports*



1. Investigating cells

2. Uncapping of cells

3. Recapping of cells

4. Removal of brood



New Breeding Program

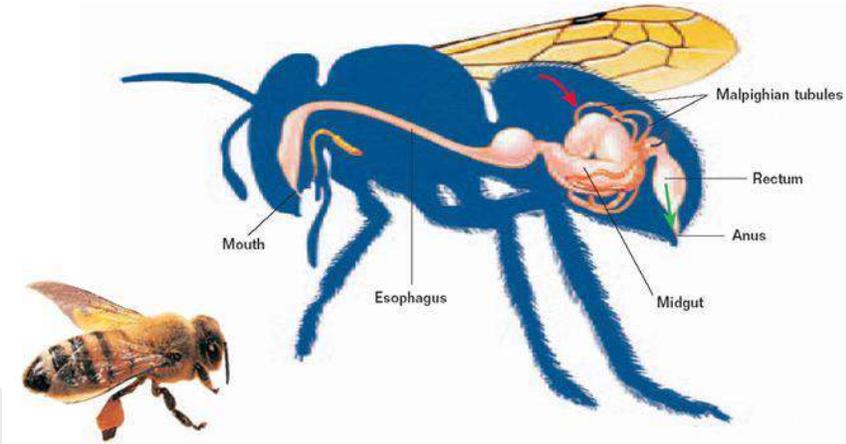
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Care strategies
in honey bees?

Honey Bee Care

- Grooming (Peng et al. 1987)
- Venom (Baracchi et al. 2011)
- Antimicrobial secretions in brood food/ royal jelly (Fujiwara et al. 1990; Klaudiny et al 1999)
- Microbiota in guts and stored pollen
- Propolis contact and volatiles in nest cavity



CARE: Hygienic behavior and chalkbrood disease



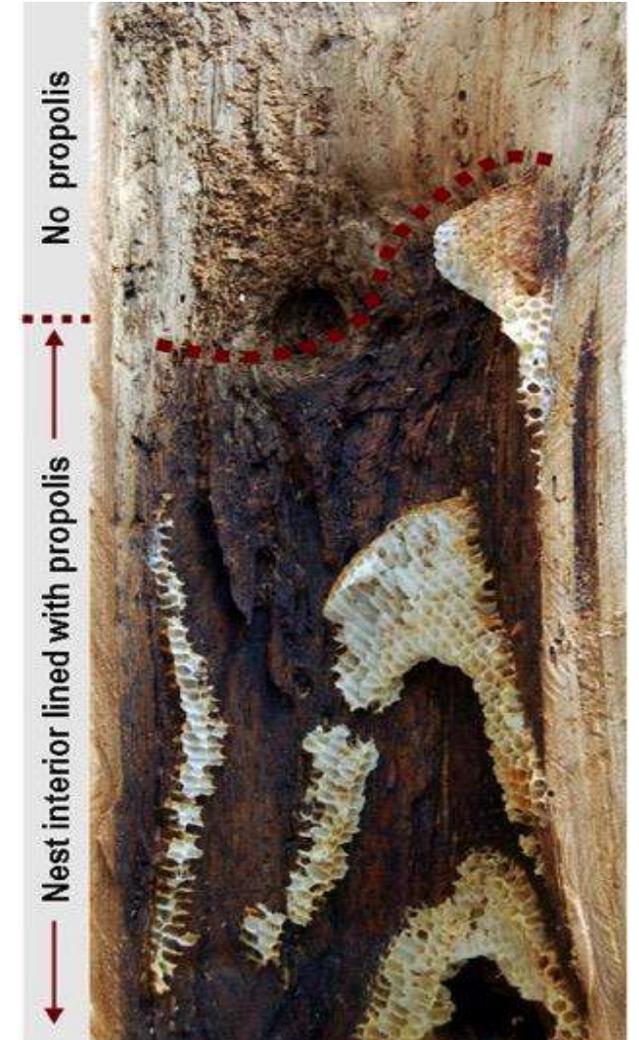
Chalkbrood
Ascosphaera apis

Gilliam, Taber et al. 1983

- “Hygienic behavior [is] the primary mechanism of resistance to chalkbrood, although resistance involves other factors as well.”
- Bees add antagonistic molds and bacteria from microbiome (primarily *Bacillus* spp.) to pollen that inhibit *A. apis*.
- Bee colonies that are resistant have more of these antagonists.

CARE: Resin = Propolis is highly antimicrobial

Propolis collection evolved as mechanical barrier, and in response to fungi and bacteria in tree cavity



Benefits of Propolis Envelope

- Propolis envelope benefits bees' immune system
- Propolis envelope helps reduce disease loads
- Bees collect more resin after infection with chalkbrood = social medication
- Bees in our area collect resin mostly from cottonwood trees: *Populus deltoides*
- Propolis exposure and microbiome (unpubl)



Reviews:

Simone-Finstrom and Spivak, 2010. *Apidologie*

Simone-Finstrom et al 2017. *Insects*

Spivak et al, 2019. *Curr. Op. Ins. Sci.*



Selecting for Health: Care and Kill Strategies

- Winter survival
- Good spring build up
- Care: Propolis collection
 - traps and rough inner walls
- Kill: Hygienic behavior
 - freeze-killed brood
 - chemical assay
 - marker- assisted (Guarna et al, 2017 CANADIANS!)
- Virus load
 - Declan Schroder - virologist

