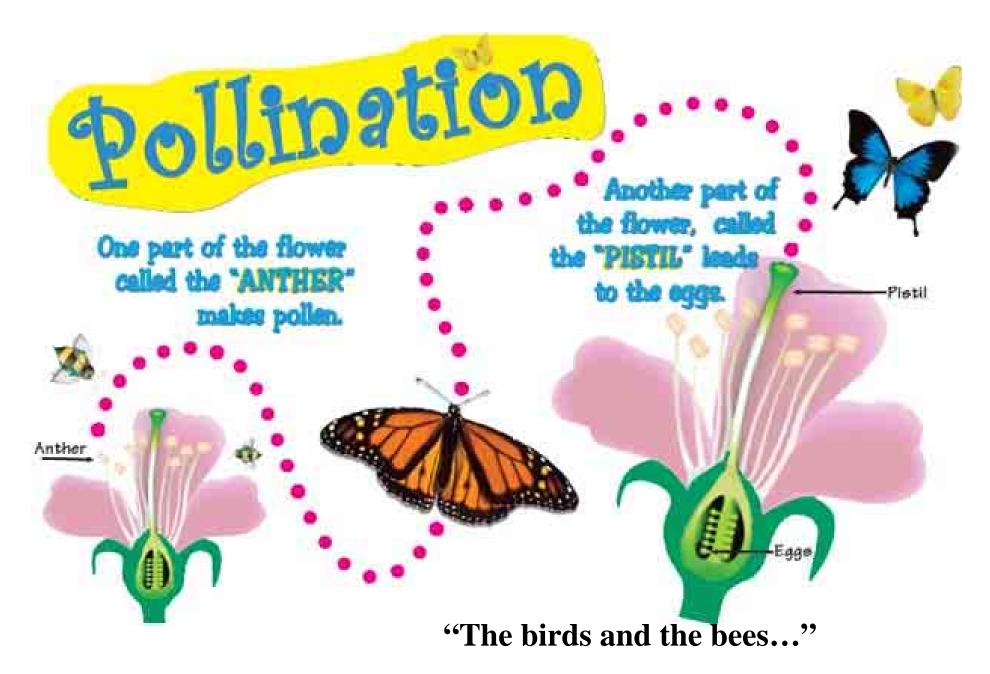




Bees—internationally recognized for their role in pollination...



http://kidsgrowingstrong.net/images/pollination2.jpg

#### **Pollination**

--the process by which pollen grains, containing male sex cells, are transferred to stigmas, female floral parts, to bring about fertilization, a necessary step in producing seeds

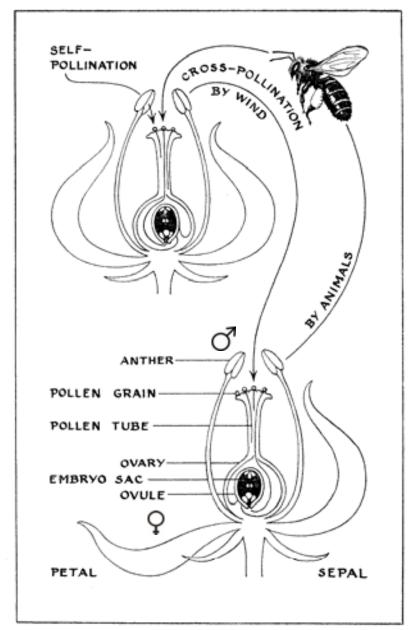


Figure 1. Diagram of self- and cross-pollination.

5



For about 75% of the planet's **240,000**+ species of flowering plants, the process depends on animal partners, which deliver pollen with greater precision than wind, water, or other abiotic agents



Sexual reproduction generates variation, which is essential for survival in a changeable world. It's the main reason flowering plants (with their pollinator partners) dominate most terrestrial communities

Given that more than 400,000 species planet-wide are engaged in this process, it's remarkable how little is known about it.

Humans have been blissfully unaware of their dependence on pollinators for most of their existence on the planet; as ecological interactions go, animal-mediated pollination is rare, rapid, and easily overlooked





# Plant requirements for survival

- Sunlight
- Water
- Nutrients
- Protection from pests
- Pollination (for 75%)



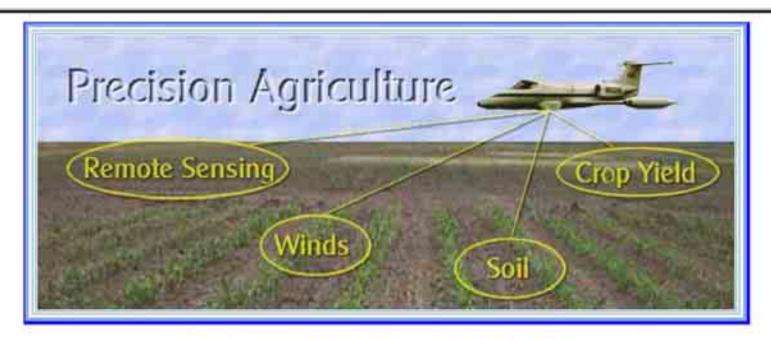
# History of agricultural technology

- Fertilization: 10,000 BCE (manuring)
- Irrigation: 6,000 BCE (Mesopotamia)



• Chemical pest control: 400 BCE (Theophrastus)





1 Remote Sensing | Winds | Soil | Crop Yield | GHCC Home |

What is precision farming?

Knowing and caring where you are in a field.

What can precision farming do for me?

- Improve Crop Yield.
- Provide information to make better management decisions.
- · Reduce chemical and fertilizer costs through more efficient application.
  - Provide more accurate farm records.
    - Increase profit margin.
      - Reduce pollution.

# History of agricultural technology

- Fertilization: 10,000 BCE (manuring)
- Irrigation: 6,000 BCE (Mesopotamia)



• Chemical pest control: 400 BCE (Theophrastus)

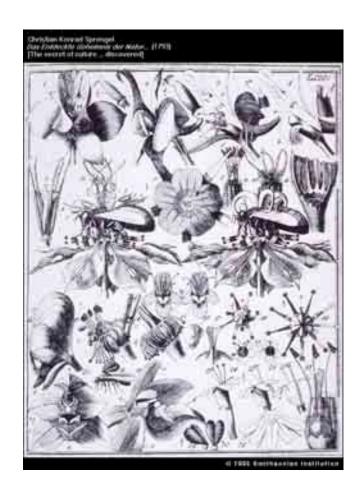


• Pollination: 17th century (R. J. Camerarius)

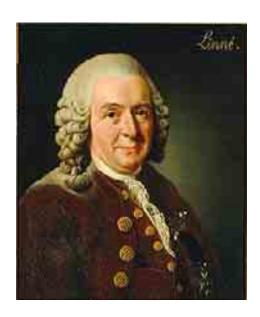




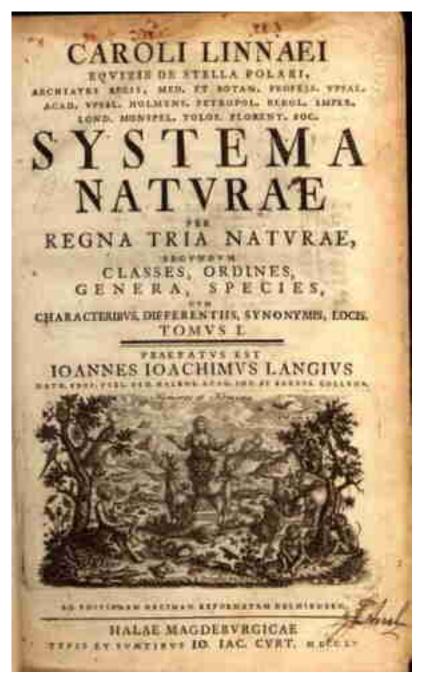
Although honey bees have been semi-domesticated for thousands of years, management for the purpose of pollination delivery is a post-Enlightenment phenomenon







That plants have sexual organs is a relatively new concept. Carolus Linnaeus 1707-1778 used the sexual organs of plants to devise a system of classification and shocked his contemporaries.
The Reverend Samuel Goodenough, Bishop of Carlisle remarked, "To tell you that nothing could equal the gross prurience of Linnaeus's mind is perfectly needless"



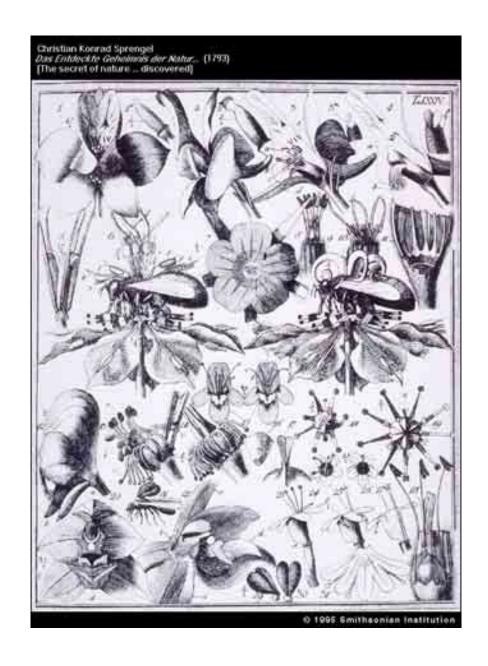
Joseph Gottlieb Kölreuter (1733-1806), professor of natural history at the University of Karlsruhe, Germany

Kölreuter was the first to demonstrate that insect visitation is necessary for seed production in many important fruits, vegetables, and ornamental flowers. He applied his knowledge by developing techniques for artificial fertilization and by conducting the first cross-hybridization of two plant species



# Christian Konrad Sprengel (1750-1816)

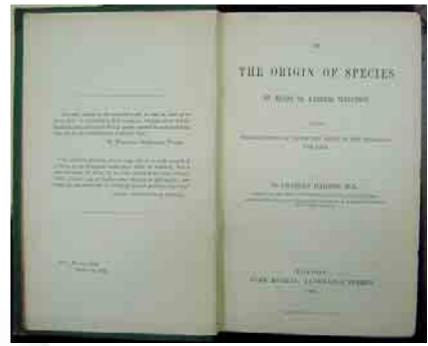
C.K. Sprengel 1793, Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen (followed by Die nützlichkeit der bienen und die nothwendigkeit der bienenzucht, von einer neuen seite dargestellt)



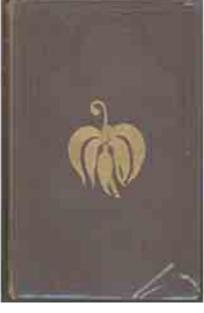
http://www.sil.si.edu/Exhibitions/Science-and-the-Artists-Book/biohlstm

Darwin C. 1859. On the Origin of Species by Means of Natural Selection. London: Murray. 365 pp.

("coadaptations of organic beings to each other and to their physical condition of life")

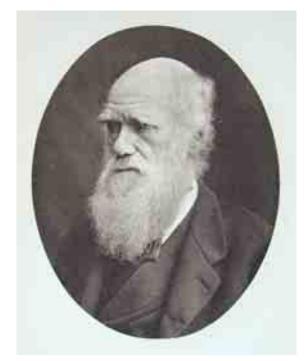








Darwin C. 1862. On the Various Contrivances by Which British and Foreign Orchids Are Fertilized. London: Murray. 365 pp.



# Attributes of honey bees conducive for use as managed pollinators

Large colony size for servicing extensive monocultures

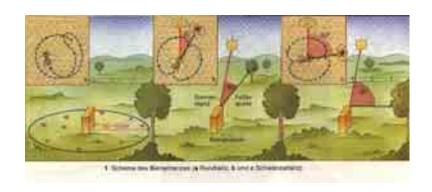
Elaborate communication system to promote flower fidelity

**Extremely broad diet** 

Ability to learn to handle many kinds of flower types

Cavity-nesting habit well-suited to management purposes

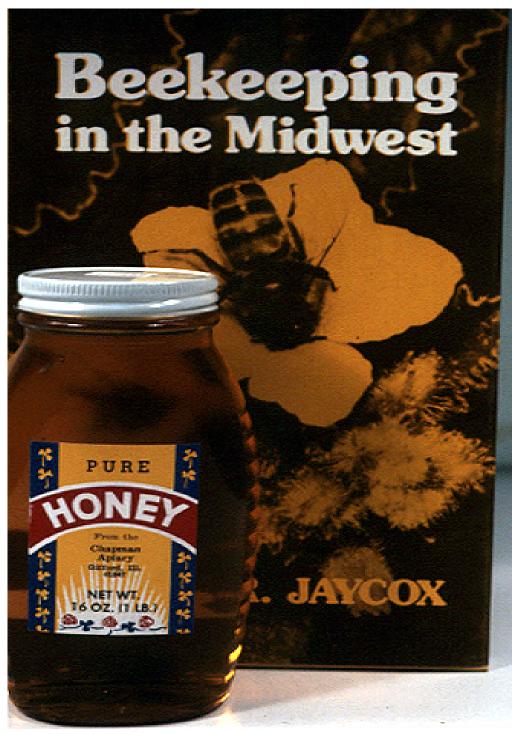








Today, honey accounts for only about 25% of the value of the apiculture industry in the United States



# Pollination by honey bees--a 15+ billion dollar service to US agriculture (>90 crops)

- Direct result:almond, apple, avocado, blueberry, cantaloupe, cherries, cranberries, cucumber, citrus, plums, peaches, pumpkin, strawberries, watermelon, zucchini
- Indirect result: dairy products (from alfalfa and clover hay), seeds (carrots, celery, onion), higher yields (peanuts, soybeans, olives, grapes)

# Portfolio effect

• diversification of holdings minimizes risk and volatility



Photo: MAAREC

# Decline in commercial beekeeping in the United States

- 1976: number of beekeepers estimated at 212,000 (U.S. International Trade Commission)
- 1991: number of beekeepers estimated at 139,000 (Bee Culture Magazine)
- 1992: number of beekeepers estimated at 125,000 (Bee Culture Magazine)



Africanized bees (1990)



Varroa mite (1987)



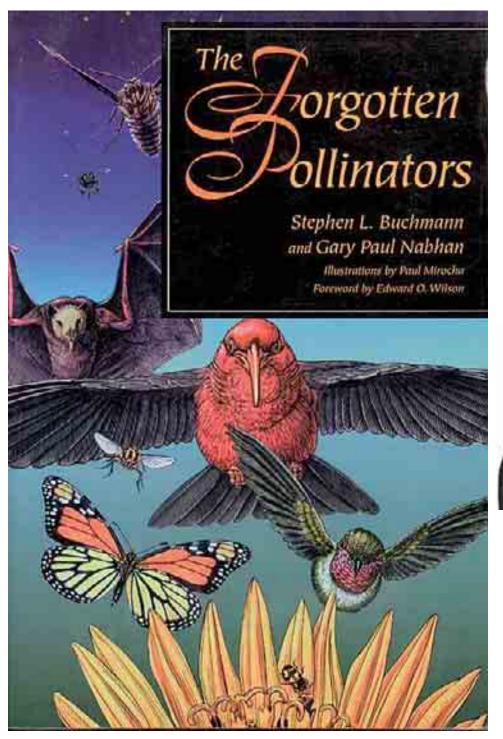
Tracheal mite (1984)



Small hive beetle (1998)

Feral bees were hard-hit but just how hard-hit is impossible to determine, given the general lack of survey data





The Forgotten Pollinators," by S. L. Buchmann and G. P. Nabhan, 1996





# Pollinator policy efforts

- September 1996 Subsidiary Body on Scientific Technical and Technological Advice of Convention on Biodiversity, **Montreal**, to establish an "international pollinator conservation initiative"
- November 1996, Third Conference of the Parties to the Convention on Biodiversity, **Buenos Aires** Decision III.11, pollinators are "priority group"
- October 1998, International Workshop on Conservation and sustainable use of pollinators in Agriculture, **Sao Paolo**--Declaration
- January 1999 Systematics Society of **Southern Africa**, African Pollinator Initiative
- 1999, North American Pollinator Protection Campaign, Coevolution Institute
- May 2000, **Keny**a, Fifth meeting of COP, International Initiative for the conservation and sustainable use of pollinators--FAO invited, International Pollination Initiative
- April 2002, IPI approved at COP6, **The Netherlands**
- 2004, National Research Council study approved, with USDA/USGS funding







# Status of Pollinators in North America Report Briefing October 2006

•May Berenbaum (chair)

•Peter Bernhard, St Louis University'

•Stephen Buchmann, The Bee Works

•Nicholas Calderone, Cornell University

•Paul Goldstein, Florida Museum of Natural History

•David Inouye, University of Maryland

•Peter Kevan, University of Guelph

•Claire Kremen, University of California-Berkeley

•Rodrigo Medellin, University of Mexico

•Taylor Ricketts, World Wildlife Fund

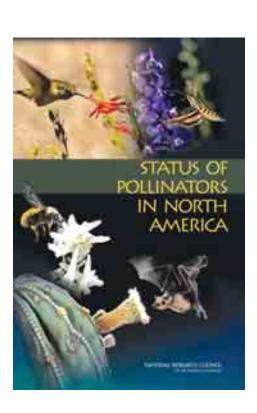
•Gene Robinson, University of Illinois Urbana-Champaign

•Allison Snow, Ohio State University

•Leonard Thien, Tulane University

•F. C. Thompson, U.S. National Museum

•Dr. Scott Swinton, Michigan State University

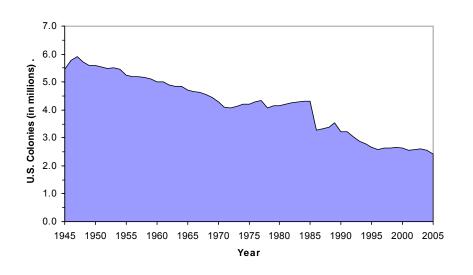




# Managed Pollinators

#### Status

- Long-term population trends for honey bee in the United States are demonstrably downward.
- Similar data are not available for other managed pollinators.



U.S. honey bee colonies, 1945-2005. Data compiled from USDA-NASS



### Causes of decline

#### Managed and wild species

- Introduced pathogens and parasites
- Habitat degradation and loss

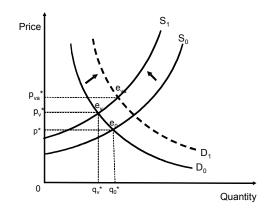


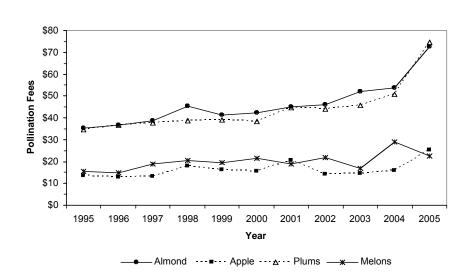


## Managed Pollinators

## Consequences of Decline

Declines in honey bee supplies in the absence of viable alternative species are exacerbated by increasing demands for pollination services, creating shortages and raising pollination costs





Fall Dwindle Disease: A preliminary report December 15, 2006



"Fall-Dwindle Disease": Investigations into the causes of sudden and alarming colony losses experienced by beekeepers in the fall of 2006.

#### Preliminary Report: First Revision

Dennis vanEngelsdorp<sup>12</sup>, Diana Cox Foster<sup>2</sup>, Maryann Frazier<sup>2</sup>, Nancy Ostigny<sup>2</sup>, Jerry Hayes<sup>3</sup>

December 15, 2006 Revised January 5th, 2006

During the months of October, November, and December 2006, an alarming number of honey bee

colonies began to die along the East Coast of the United States. West Coast beekeepers are also beginning to report unprecedented losses. This phenomenon, without a recognizable underlying cause, has been tentatively been termed "Fall Dwindle Disease", and threatens the pollination industry and production of commercial honey in the United States. This has become a highly significant yet poorly understood problem for beekeepers. States, like Pennsylvania, can ill afford these heavy losses; the number of managed colonies is less than one half of what it was 25 years ago. Many beekeepers are openly wondering if the industry can survive. There are serious concerns that losses are so great that there will not be enough bees to rebuild colony numbers in order service pollination needs and to maintain economic viability in these beekeeping operations.

Also in October, 2006, reports of mysterious honey bee disappearances began to surface...

# Colony Collapse Disorder (CCD)



- -- November 2006 Dave Hackenberg lost 400 colonies in Florida
- -- Other beekeepers experienced similar sudden losses

# A new phenomenon?

- -- Bees die away from the hive
- -- Queen, grubs, food left behind
- -- Scavengers slow to move in



Keith Delaplane

#### Many Bee Colonies Dead of an Unknown Cause

by E. CERTEL Internal of Principles and Principles

The term "spring dwindling" used | keeper of Arcpelles Parish, La., reto be seen frequently in beckeeping literature. It related to the rapid began between October 15 and Nodeath of the bees in a colony in the vember 1, 1963. A besisener in Asspring in Northern States until all or nearly all of them were dead. Boxt (1975) discussed apping dwin-ding at some length. He also used the term "winter deciding." In the 14st term "winter deciding." In the strike the loss of less that socurry the Agricultural Extension Division, is the witter months in senitropical Cittales. Various causes were given attempt was made early in February Toll the decline or death of colonies: lew vitality, dynestery, poor food, lack of protection, sld bees, or lack of

# Bulley (1963) discussed the or casional heavy losses of honey bee colonies reported in England since 1905. Although most of the losses were said at the time to have been second by the his of Wight disease. Bulley believed that the louses were ainly the result of other factors lack of food, neglect, unfavorable weather, poor beckeeping, or foulbrood. He stated, "Recent cases of arge scale losses of born in auturen in Peruguap, Northern Argentina and Brazil are of been unable to fly, with their abdomens distended, crawling away from their hiven." Balley supported that poleonous nectar or Andrew was breedend

The following report is concern with the less of many colonies of bous in the fall of 1963 and winter of 1963-64. Descriptions from becomes and a study of the dead or dying colonies, the honey, and the pollen indicate that we are concerned with neither "winter dwindling" nor polsonous honey. If a name is needed, dulading" could be used.

Thousands of colonies died in south central Lephana and northeast Tenas (see man) and smaller numbers in a small area routh of Houston, Texas, and undetermined numbers in California, Arisona, New Mexico, and Alabama. A Leuisiana man with 15 years of beckeeping exserionce stated that he had never before seen anything like it. A bee-

Trum correspondence of B. Cartino, existing State Entereshiplet, with State bloominglets and Aphenius, and kindly used in this writer.

ported that he believed the tecension Parish first noticed losses on November 1. Combs and bees from at Louisiana State University\*, an 1964 to leave how many enhance it Louisiana died during the full of 1903 and the winter of 1963-64. Some beekeepers did not answer the inquire. some reported no looses, and others reported that 50 per cent of their colenies had died. Some lost several bundred colonies and it is believed that around 1,000 colonies were lost by at least one beckeeper. Probably between 4,000 and 5,000 colonia either died or become extremely weak in a relatively small area in Leuisiana, Reliable reports from northeast Tenas suggested that much the same situation occurred there. We can be sure that several thousand colunles died within a period of about 3

Members of our laboratory visited beckeepers and aplayee in south central Louisiana in an effort to de-

W. A. Cancionna, Assistant Specialist In



Dr. E. Ownell

termine what exceed the death of c colonies. Colonies were normal some aglaries while in a mile or two away a few to m were already dead or had only a conviving best tiln some cases than a curful and a core parently the worker born died field because only a few dea were the survivors, rather than victims. We did not see the colo in the northeast Texas area, but w formed by reliable observers t conditions there were like those the affected area in Louisiana.



Oertel, 1965, American Bee Journal

Strange Administ Wigner Out Colomors of Beer ADMAN SAME, 70% Landgelor Times (1986 Commer File): New 21, 1995, ProQuert Missocial Newspapers Lee Augulies Times (1985 - 1995)

#### Strange Ailment Wipes Out Colonies of Bees

Laboratories at Work on 'Autumn Collapse' Affecting State's \$5 Million Honey Crop

#### 'BY ADRIAN HAMILTON

They call it 'autumn col- ment of Agriculture officials lapse" and it's got the ex- have got together with beeperts puzzled in laboratories keepers agricultural commisat Berkeley, Beltsville, Md., stoners, farm, advisors and and Lazamie, Wyo. A bee the USDA to thoroughly inmalady, it hits only certain vestigate the disorder. areas and then only in the Monitoring traps to catch fall. But, in those apiaries sick and dead bees have 90% of the colony.

denly start to decline for no County. At the same time apparent reason during an scientists at USDA Bee turn and collapse within six Disease Labs at Beltsville, months, Len Foote, supervi-sor of apiary inspection for the Inversebrate Pathology the State Department of Lab at Berkeley, are con-Agriculture, told a conven-ducting intensive tests of tion of state beeksepers at bees, brood, pollen, water, Sacramento last week.

California Departments of rasites. Agriculture are giving high Agriculture as group and priority to identifying and Though no reports of the quin and Impecial Vallays, controlling the malady be-disorder came in from other Laboratory reports are excause of the threat to honey states, the California moni- pected in the near future and crop production.

"The value of honey pro- mid-October. "The varie of nonly pos-duction in California is Co-operating beekeepers in the same counties also but we are hopeful," said froste said, "but altogether found signs of autumn col-line froste." This year it isn't about \$300 million of crops lapse in their aplacies. La-as widespread as last. We depend on honey bee pollina-bersurey tests eliminated all know at least that the disco-

plums and also the seeds of answer.

Tests have shown that the contact their county agriculas foraging for dairy cattle possibility of pesticide being tural commissioner or farm as well an vegetable seed and responsible to extremely un-almost all of the melon crops. likely and that it is probably of this disorder in their apia-In addition Sacramento not an infectious disease.

been attached to selected co-"strong colonors, neavy lordes in Glern County, Ta-with honey and pollen, sud. hama County and Shasta nectar and honey for possi-Officials of the U.S. and ble textus, pathogens or pa-

#### . Colonies Munitared

tored colonies had reached and in January officials and Worth \$5 Million a Year the final stages of collapse by scientists will get together to

tion for their production.\* known bee diseases and pa-der is not transmittable. But The bees pollinate fruit rasites but, up to now at any we need to know how widecrops like apples, prunes and rate, have come up with no spread it is and would urgo

white same 200 tons of ... Attampts to euro orienter

evaluate their findings.

Disappearances have been reported from time to time but Colony Collapse Disorder appeared to be qualitatively and quantitatively different ...



By February, massive disappearances had occurred in over 20 states...

Beekeepers and researchers convened a meeting in Florida to discuss the possible causes and consequences

http://www.doacs.state.fl.us/pi/plantinsp/apiary/images/ccdstates.jpg



USDA bee researchers collected samples of afflicted bees in Pennsylvania and Florida in order to figure out what was causing the problems...

#### REVIEW COLONY COLLAPSE DISORDER IN HONEY BEE COLONIES ACROSS THE UNITED STATES

#### HEARING

BURGES THE

SUBCOMMITTEE ON HORTICULTURE AND ORGANIC AGRICULTURE

#### COMMITTEE ON AGRICULTURE HOUSE OF REPRESENTATIVES

ONE HUNDRED TENTH CONGRESS

PERT SERVICE

MARCH 29, 200

Serial No. 110-07



Printed for the use of the Committee on Agriculture agriculture Assess, per

EA COMMOMENT PROPERCY OFFICE

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#### Majority

- \* Dennis A. Cardoza, C Chairman
- \* Bob Etheridge, NC
- \* Lincoln Davis, TN
- \* Tim Mahoney, FL
- \* John Barrow, GA
- \* Kirsten E. Gillibrand, NY

#### Minority

- \* Randy Neugebauer, TX Ranking Minority Member
- \* John R. "Randy" Kuhl, NY
- \* Virginia Foxx, NC
- \* K. Michael Conaway, TX
- \* Robert E. Latta, OH



Richard Adm, left and Jim Doan teatify before the lisuse Subscrending on Horizothere and Organic Agriculture

May 2007 BEE CULTURE

The extensive losses caught the attention of legislators in key states (California, with its \$2.5 billion almond industry, and Florida) and the House Agriculture Committee Subcommittee on Horticulture and Organic Agriculture (which oversees apiculture) held a hearing on Colony Collapse Disorder on March 29, 2007

RACHESCHIEF STEET ENGLISH EMPERALAS FORCE WITHIN WITH

Front Page 1 World | Europe | Gretany 1 Berinson | Zeitgeist | International |

English Size > World

March 72, 2007

Front I fi Hall | Treedback I Share

COLLAPSING COLONIES

First: 122

#### Are GM Crops Killing Bees?

By Gunther Latsch

A mysterious decimation of bee populations has German beekeepers worried, while a similar phenomenon in the United States is gradually assuming catastrophic proportions. The consequences for agriculture and the economy could be enormous.



Is the mysterous decimation of time populations in the US and Germany a moult of GM crops? Walter Haefeker is a man who is used to painting grim scenarios. He sits on the board of directors of the German Beekeepers Association (DBIB) and is vice president of the European Professional Beekeepers Association. And because griping is part of a lobbyist's trade, it is practically his professional duty to warn that "the very existence of beekeeping is at stake."

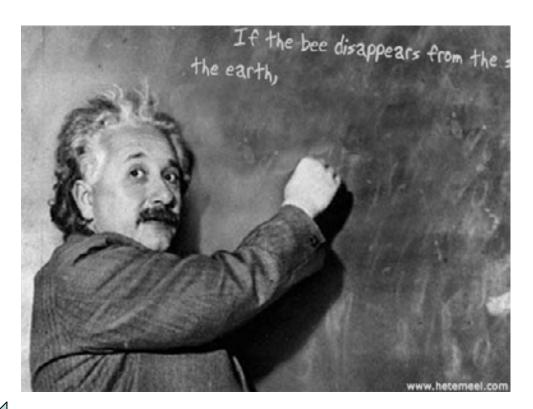
The problem, says Haefeker, has a number of causes, one being the varros mits, introduced from Asia, and another is the widespread practice in agriculture of spraying wildflowers with harbicides and

practicing monoculture. Another possible cause, according to Haefeker, is the controversial and growing use of genetic engineering in agriculture.

As far back as 2005, Haefeker ended an article he contributed to the journal Der Kritischer Agrarbericht (Critical Agricultural Report) with an Albert Einstein quote: "If the bee disappeared off the surface of the globe then man would only have four years of life left. No more bees, no more pollination, no more plants, no more animals, no more man."

Mysterious events in recent months have suddenly made Einstein's apocalyptic vision seem all the more topical. For unknown reasons, bee populations throughout Germany are disappearing — something that is so far only harming beekeepers. But the situation is different in the United States, where bees are dying in such dramatic numbers that the economic consequences could soon be dire. No one knows what is causing the bees to perish, but some experts believe that the large-scale use of genetically modified plants in the US could be a factor.

http://www.spiegel.de/international/world/0,1518,473166,00.html



"Auf einen Satz verweisen betroffene Bienenzüchter und nüchterne Bienenforscher inzwischen immer wieder, einen Satz, den Albert Einstein einmal gesagt haben soll: "Wenn die Biene von der Erde verschwindet, dann hat der Mensch nur noch vier Jahre zu leben; keine Bienen mehr, keine Bestäubung mehr, keine Pflanzen mehr, keine Tiere mehr, keine Menscheß6

mehr...". ...

#### CCD Meeting @ USDA BARC, April 23-24, 2007



On April 23-24, about fifty bee researchers and other interested parties convened at a workshop at USDA BARC to prioritize Colony Collapse Disorder research objectives

# Hypotheses to account for colony collapse disorder Most likely Less likely

- Neonicotinoid insecticides
- Novel pathogen or parasite
- Immune suppression relating to management practices
- Declines in nutritional adequacy of diet
- NH NH

- GM corn pollen
- Cell phones
- Wi-Fi
- Elevated carbon dioxide
- Elevated UVB light
- Osama Bin Laden
- Automobile grilles
- Solar maxima
- Jet chemical contrails
- Fluctuations in the Earth's magnetic field
- Alien abduction
- Bee "rapture"

Imidacloprid

#### **CRS Report for Congress**

**Recent Honey Bee Colony Declines** 

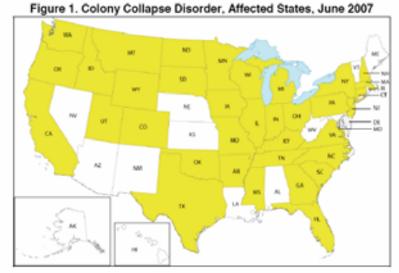
Updated June 20, 2007

Analyst in Agricultural Economics Resources, Science, and Industry Division

On June 20, 2007, the Congressional Research Service updated its report on Colony Collapse Disorder

By this point, CCD had been reported in 35 states

Renée Johnson



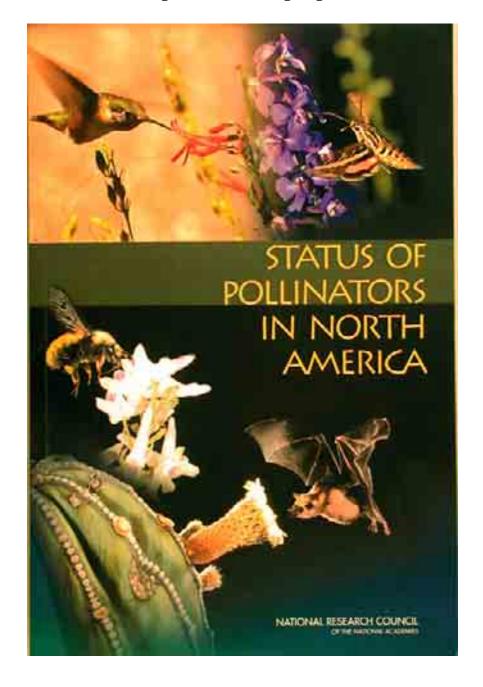
CRS-5

Source: Bee Alert Inc., [http://www.beealert.info/]. Shaded areas show reported affected states.

Prepared for Members and Committees of Congress



and the NAS report seemed prophetic...



"The U.S. commercial honey bee population was stable from 1996 to 2004, but if it were to continue to decline at the rates exhibited from 1947 to 1972 and from 1989 to 1996, it would vanish by 2035" (p. 118)



The original Langstroth hive.

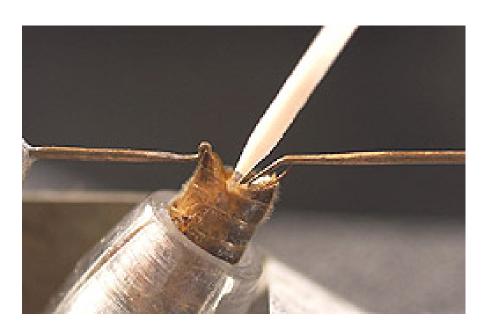


Beekeeping technology is for the most part unchanged since the 19th century. For an industry that contributes about \$15 billion annually, it's remarkably unimproved





Chief 20<sup>th</sup> century technological innovations in beekeeping include rubber tires and airconditioning for trucks and artificial insemination instrumentation



http://www.ars.usda.gov/



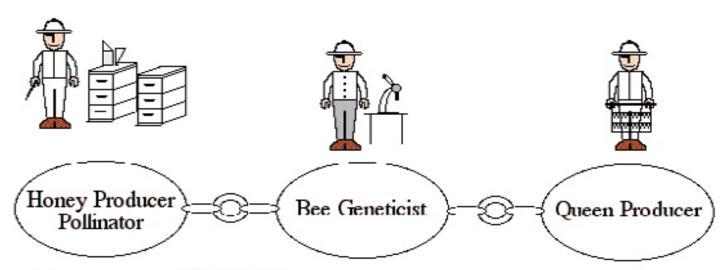
A line of honey bees resistant to mites (SMR, "suppressed mite reproduction" or VSH, "Varroa-sensitive hygiene" bee), was selected but it's not commercially available...



To demonstrate Varroa-sensitive hygiene by SMR bees, a highly infested brood comb was cut into halves, and each half was placed in a cage with 2,000 test bees for 24 hours. Shown here is the brood comb of the SMR bees, which removed 215 pupae and uncapped another 178 pupae (90 percent of uncapped cells were infested with Varroa mites).

(D214-2)

#### Links in the Chain of People and Tasks Necessary to Supply Better Bees to the Beekeeping Industry



Task: Carry out field tests where stock is to be used. Make plans:

- On how to get improved stock.
  - a. Decide how to do field tests
  - b. Select colonies to breed from
  - c. Decide when to use natural mating or artificial insemination
- On how to maintain improved stock over the years.
- On how to release improved stock.

Produce improved stock for sale to honey producers, bee producers, and/or pollinators.



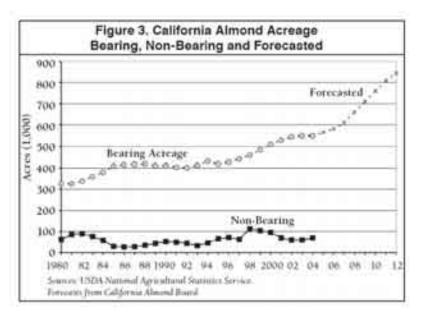
Increasing demand for pollination services is pushing the limits of the system in an unprecedented way

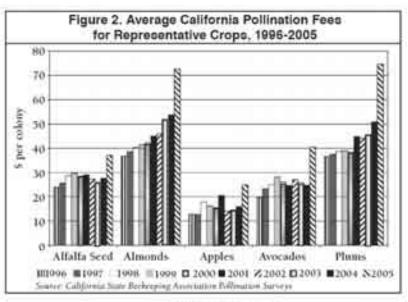






http://www.indiansummerhoneyfarm.com/slide1.jpg http://boxer.senate.gov/news/photos/features/farmbill/ If almond acreage continues to expand, by 2012 every honey bee in America will be needed to pollinate just that crop; however, demand for pollination services is increasing for other crops as well.





Bee-conomics and the Leap in Pollination Fees by Cantel A. Screens and Highly Borise Contributions of native pollinators is estimated to be worth \$3 billion but prospects of expanding the use of native pollinators are dim





Clean weed-free fields without fences have no nectar or nesting resources <a href="http://www.seaburst.com/cornfield01.jpg">http://www.seaburst.com/cornfield01.jpg</a>

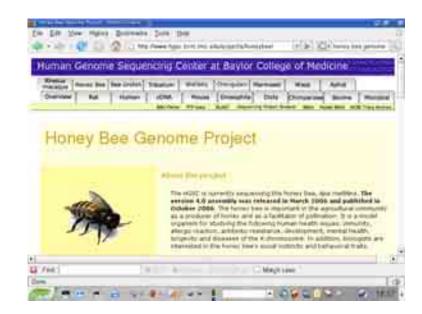
# Where have the bees gone?



# A new forensic tool for understanding CCD: the honey bee genome (October 2006)



- -- use genome-derived tools to find out what's happening
- -- or at least find good diagnostics for determining if a colony has CCD or not



# Insights into social insects from the genome of the honeybee Apis mellifera

The Honeybee Genome Sequencing Consortium\*

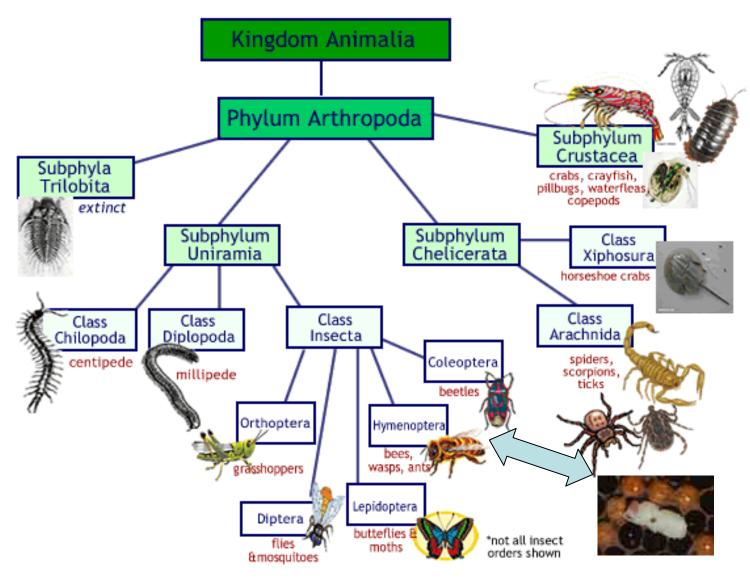
Here we report the genome sequence of the honeybee *Apis mellifera*, a key model for social behaviour and essential to global ecology through pollination. Compared with other sequenced insect genomes, the *A. mellifera* genome has high A+T and CpG contents, lacks major transposon families, evolves more slowly, and is more similar to vertebrates for circadian rhythm, RNA interference and DNA methylation genes, among others. Furthermore, *A. mellifera* has fewer genes for innate immunity, detoxification enzymes, cuticle-forming proteins and gustatory receptors, more genes for odorant receptors, and novel genes for nectar and pollen utilization, consistent with its ecology and social organization. Compared to *Drosophila*, genes in early developmental pathways differ in *Apis*, whereas similarities exist for functions that differ markedly, such as sex determination, brain function and behaviour. Population genetics suggests a novel African origin for the species *A. mellifera* and insights into whether Africanized bees spread throughout the New World via hybridization or displacement.

Table 3	Gene fam	ilv size differe	nces with pos	ssible effects	on honevbee	lifestyle
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Family	Function	Family compared with Drosophila	Possible lifestyle effects			
Major royal jelly	Brood feeding	Larger	Brood care; caste development <sup>92</sup>			
Insulin/insulin-like growth factors	Ageing, fertility, many others	Variable for different subfamilies	Unique reversal of typical lifespan/fertility trade off			
Cuticular proteins	Cuticle stability	Smaller	Protected hive environment allows simpler cuticle			
Odorant receptors	Olfaction	Larger	Enhanced pheromone communication; odour-based			
		_	kin recognition; generalist flower feeder			
Gustatory receptors	Gustation	Smaller	Brood feeding: mutualistic flower feeder reduces			
			threat of toxic food			
Immunity	Infectious disease protection	Smaller	Paradox: high pathogen load due to sociality			
Detoxification genes	Defence against xenobiotics	Smaller	Managed environment; specialized lifestyle			



Mite management presented the challenge of finding selective acaricides that do not kill bees



itp.nyu.edu/animals/ 02/taxa.htm

## Tau-fluvalinate 615mg per Apistan strip

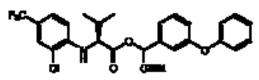
approx. 0.125 µg/bee/day

0.1 µg/bee recorded 1 yr. after treatment (Haarmann et al. 2002)







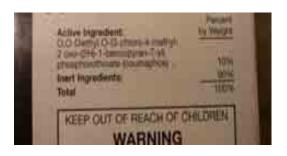


# Coumaphos

1.4g per CheckMite+ strip

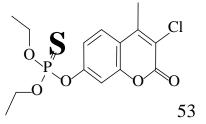
approx.  $0.33 \mu g/bee/day$ 

3.2 µg/bee recorded (Haarmann et al. 2002)□

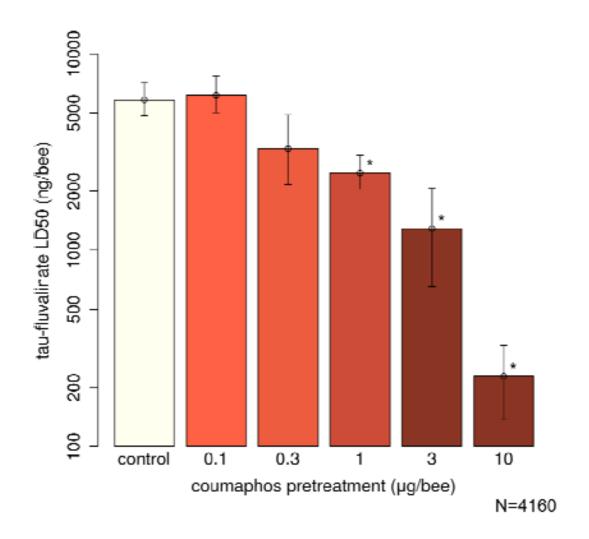




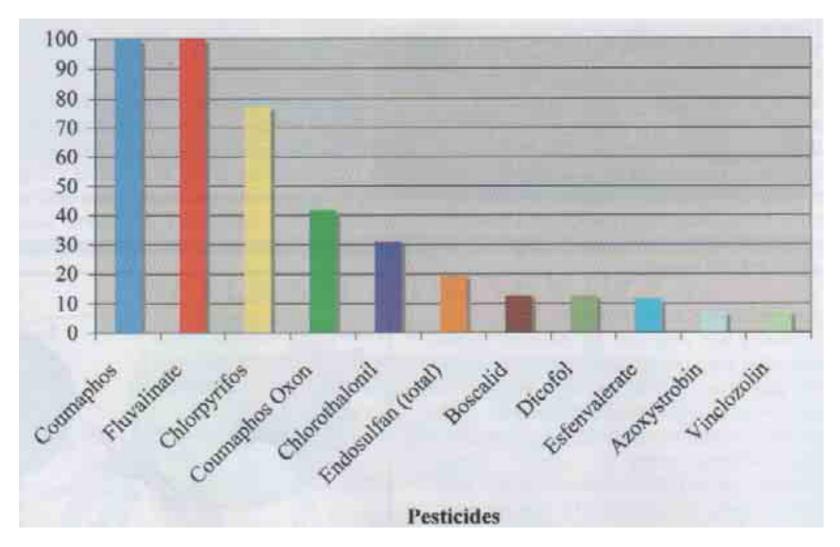




Each miticide interferes with detoxification of the other (Reed Johnson et al. 2009)







Frazier et al., 2008. American Bee Journal.

Coumaphos and fluvalinate are the most frequently detected pesticides in brood nest wax of honey bees—they were present in 100% of samples

55

In-hive pesticides

Apistan





Agricultural pesticides (particularly neonicotinoids, synthetic neurotoxic analogues of nicotine)





#### Do neonicotinoids cause CCD?

-- systemic – present in nectar and pollen

-- cause learning problems in bees exposed to high doses

-- imidacloprid banned in France for use on sunflowers ("mad bee

disease")

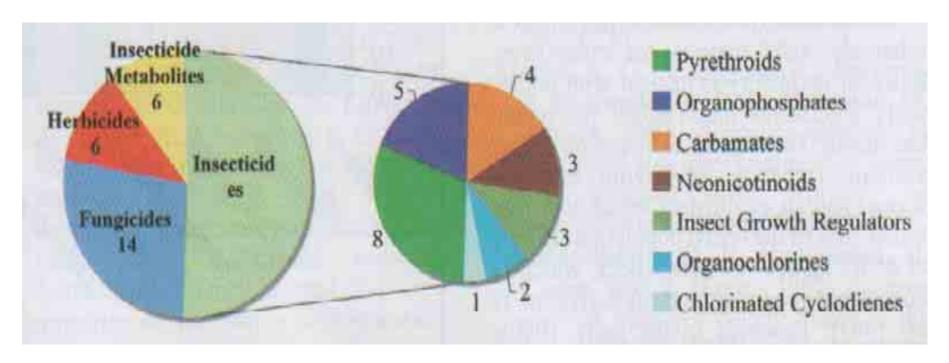






# But . . .

- -- France STILL has problems after banning imidacloprid
- -- Imidacloprid has been used in the U.S. since 1996
- -- Only 3 out of 108 pollen samples taken from CCD colonies contained imidacloprid



Frazier et al., 2008. Am. Bee J.

# Sciencexpress

#### Report

#### A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder

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

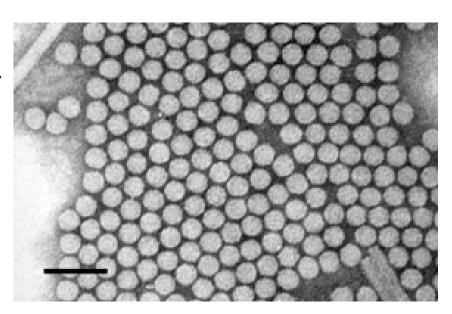
In colony collapse disorder (CCD), honey bee colonies inexplicably lose their workers. CCD has resulted in a loss of 50 to 90% of colonies in beekeeping operations across the United States. The observation that irradiated combs from affected colonies can be repopulated with naive bees suggests that infection 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 examination of samples collected from several sites over a period of 3 years. One organism, Israeli acute paralysis virus of bees (IAPV), was strongly correlated with CCD.

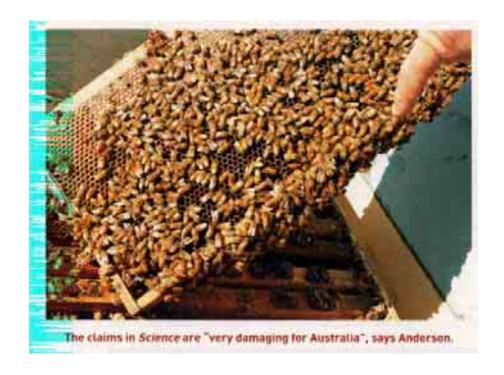
Agent		r of positive itive of samp		Positive Predictive	Sensitivity	Specificity	
1191111	CCD (n = 30)	$\begin{array}{c} \text{non-CCD} \\ (n=21) \end{array}$	Total (n = 51)	Value (%)	(%)	(%)	
IAPV	25 (83.3%)	1 (4.8%)	26 (51.0%)	96.1	83.3	95.2	
KBV	30 (100%)	16 (76.2%)	46 (90.2%)	65.2	100	23.8	
N. apis	27 (90%)	10 (47.6%)	37 (72.5%)	73.0	90.0	52.4	
N. ceranae	30 (100%)	17 (80.9%)	47 (92.1%)	63.8	100	19.0	
All 4 agents	23 (76.7%)	0 (0%)	23 (45.0%)	100	76.7	100	

Using a viral metagenomics approach, Cox-Foster et al. 2007 demonstrated that Israeli Acute Paralysis Virus is associated with colony collapse

# Israeli Acute Paralysis Virus (IAPV)

- · First described in Israel in 2004
- · Israeli version causes shivering of wings and paralysis
- · Never before reported in the U.S.
- · IAPV was reported in 83% of CCD colonies
- · IAPV was also found in Australian bees imported for almond pollination in 2005 (after Congress lifted the 1922 ban on importation)





Australians took issue with any implication that their bees were involved...



#### HISTORICAL PRESENCE OF ISRAELI ACUTE PARALYSIS VIRUS IN THE UNITED STATES

by YANPING CHEN and JAY D. EVANS\* USDA-ARS, Hee Research Laboratory, Helixville, MD 20705

High hos colony losses in the United States this past year can be attributed in past to an uncoordered syndrome teemed. Colony Callajase Distribes (CCD). An extensitive generic survey function view, Israell heater Paradysis Views (IAPV), to be attribujely unsuclated with CCD. Giving DNA negatively day phylogenesis madysis, we provide vidence that IAPV was present in U.S. been callected serveral years prior to CCD, and prior to the revent importation into the C.S. of homes have from Australia and New Lealand. While downplaying the importance of two importations for the appearance of CCD, these counts indicate an argent need to not specific strains of IAPV for their disease impacts.

I until heat on of great agreement. Intendeduce of the U.S. and world-main things and Children v. 2000. and we continued a freehold by parabaand palluggers. During the money of 2000. 1001, a next and extreme synthesis of boney has bones are observed. This syndrawn, ishered Covery Colleges Etiesday (CCDs, in although by a copul depopulation) of adult have in common, which having a schopetti stending besed of healthy be-Yet "HIS November and pre-shiften ARIS" Calumptotopen(NovalorMed) - Europy entionic toggets het roughly 25% of business sens toffend the affacts of CCD, as defined by obstactions train. and solony looses of 230% (the Bigginstory et al., 200%) belong heating at al. last submires by once, then 50% of their specifical. White-seam name to CCD have recorded in participation (William and Managales, 1979), the severity of this ensur-For a world type-protes account to testimently and commenced by

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perhaps and John the SAC and Some. teen trial in beatty bean One militing racid; was the fight providings; between Breek Ayers Paralysis, Villas (BAFY), an sentantified Disservatorable virus, and CCD SAFV was determed in 25 of 36 (81%) CCD-affected busines for collection. had unity some of 22 handley columns office. Putter of al., 200% This may see also, thank in package here imported from Australia and recover of topic party temperof from China The stantifestion of SAPV so a servity described visco for the U.S., its secondary with an important disease, and confusions for both has necessaries and tooks series. Agree 467 lad to, prompted offsets in county this wone. These effects are for pead on part and propert more break that tribations EAFT, on dimensioning marks: name for which this and of making normal care. cause placese, and a 6 delectroning whether tAPV many differ exhapmently in their



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respondence for Some

The budy substant than quantum, one witnessed house, has complete scale that in Carl Service, before that and Pennsylvation (2015 to the Street Service Service) to 1907 for the presence of 145V. The general continues of all the street Service Ser



Gathering field partyles for minkyrin

But IAPV wasn't the answer...

-- healthy colonies in the U.S. had IAPV in 2001

Chen and Evans. 2007. American Bee Journal.

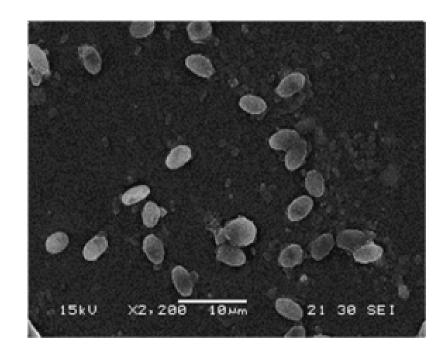
## An unusual fungal pathogen, Nosema ceranae



**MAERC** 

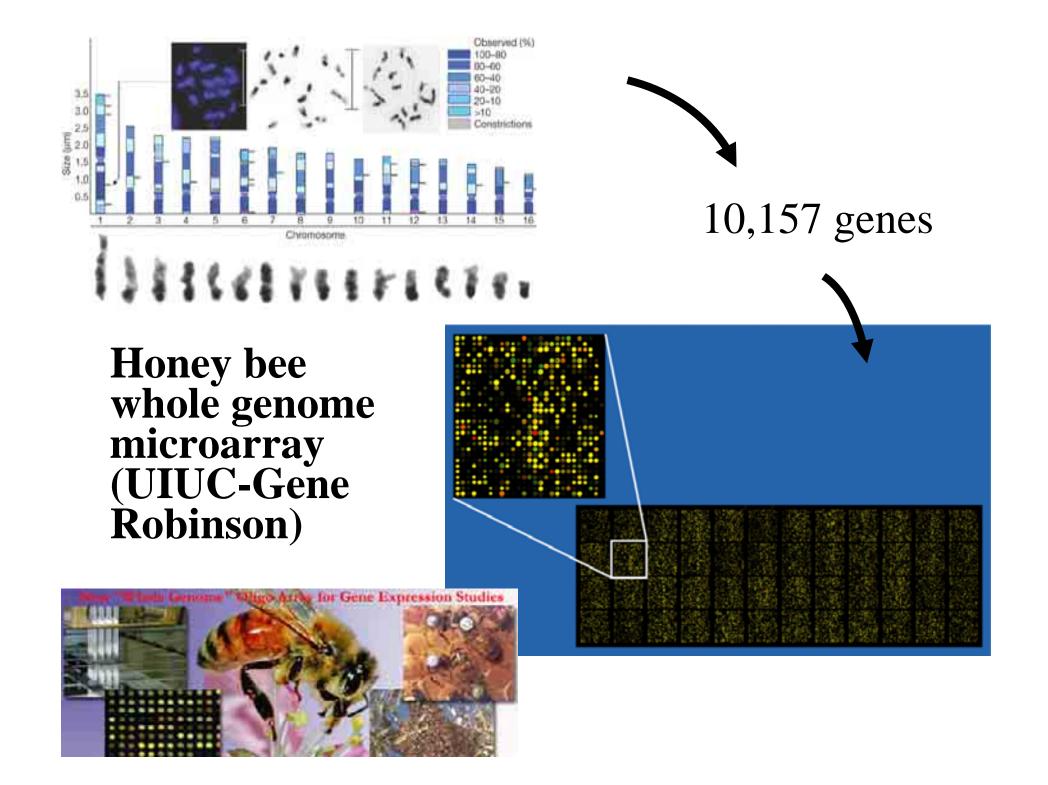
#### *N. ceranae* causes CCD?

- -- no diarrhea (in contrast with *Nosema apis*)
- --100% of CCD colonies

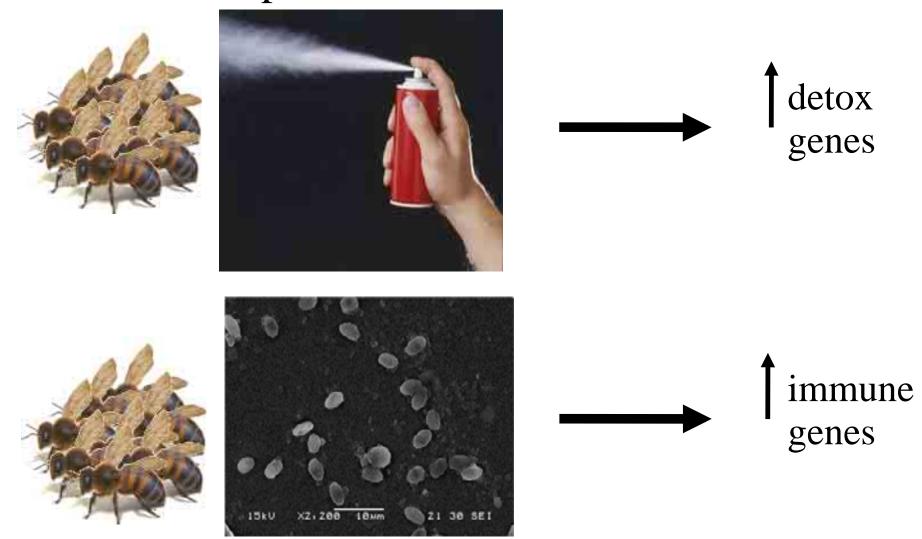


#### But...

- -- N. ceranae in US since 1995
- -- present in 81% of non-CCD colonies

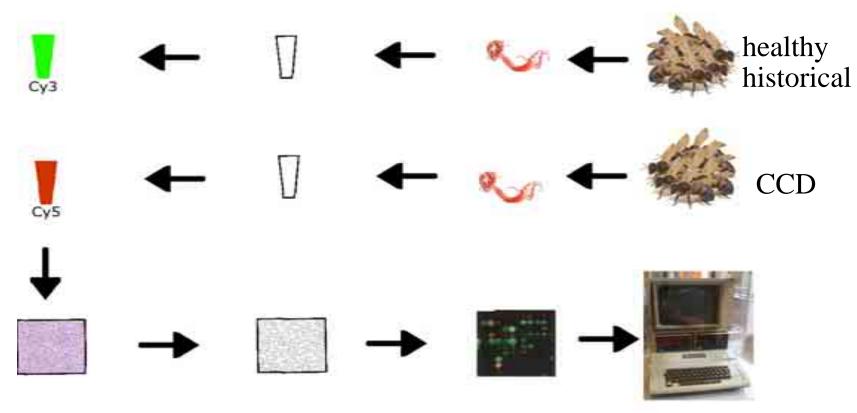


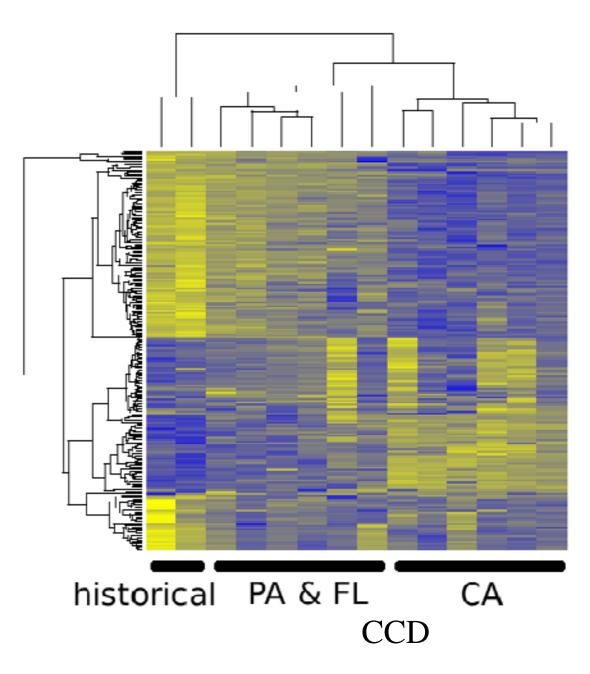
Gene expression, turning genes on or off, is a bee's first response to stress





Microarray analysis compares gene expression in "healthy" (pre-CCD) and CCD bees





Heat map displays differences between CCD and "historical" bees

Yellow--upregulated Blue--downregulated

- antibacterial proteins (apidaecins) down in CCD
  - "programmed cell death" genes up in
- **←**CCD
- "unknown" genes might be diagnostic for CCD (ribosomal RNA fragments)

#### Picorna-like viruses...

Table 3. Prevalence of pathogens in CCD and healthy bees

								Number of	Nose	ema	Number of
Year Status	State	Colonie	sABPV	KBV	IAPV	DWV	SBV	viruses	apis	ceranae	pathogens
2006 Healthy	MA/PA	14	0%	14%	7%	64%	36%	$1.21 \pm 0.80$	0%	50%	$1.71\pm0.99$
CCD	FL	24	38%	38%	25%	46%	8%	$1.54\pm1.67$	8%	42%	$2.04 \pm 2.03$
CCD	CA	57	51%	21%	21%	58%	30%	$1.81\pm1.41$	40%	60%	$2.81\pm1.74$
2007 Healthy	CA	14	86%	7%	14%	29%	7%	$1.43\pm1.02$	7%	86%	$2.36\pm1.00$
CCD	CA	16	69%	44%	25%	44%	6%	$1.88 \pm 0.72$	0%	94%	$2.81\pm0.83$

Van Engelsdorp et al. (PLoS One, 2009) also found consistently higher pathogen loads in CCD bees...

#### Colony Collapse Disorder: A Descriptive Study

Dennis vanEngelsdorp<sup>1,2</sup>, Jay D. Evans<sup>5</sup>, Claude Saegerman<sup>3</sup>, Chris Mullin<sup>2</sup>, Eric Haubruge<sup>4</sup>, Bach Kim Nguyen<sup>4</sup>, Maryann Frazier<sup>2</sup>, Jim Frazier<sup>2</sup>, Diana Cox-Foster<sup>2</sup>, Yanping Chen<sup>5</sup>, Robyn Underwood<sup>2</sup>, David R. Tarpy<sup>6</sup>, Jeffery S. Pettis<sup>5</sup>\*

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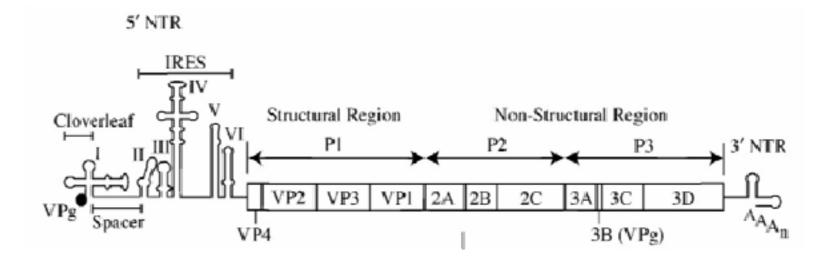
#### Abstract

Background: Over the last two winters, there have been large-scale, unexplained losses of managed honey bee (Apis mellifera L.) colonies in the United States. In the absence of a known cause, this syndrome was named Colony Collapse Disorder (CCD) because the main trait was a rapid loss of adult worker bees. We initiated a descriptive epizootiological study in order to better characterize CCD and compare risk factor exposure between populations afflicted by and not afflicted by CCD.

Methods and Principal Findings: Of 61 quantified variables (including adult bee physiology, pathogen loads, and pesticide levels), no single measure emerged as a most-likely cause of CCD. Bees in CCD colonies had higher pathogen loads and were co-infected with a greater number of pathogens than control populations, suggesting either an increased exposure to pathogens or a reduced resistance of bees toward pathogens. Levels of the synthetic acaricide coumaphos (used by beekeepers to control the parasitic mite Varroa destructor) were higher in control colonies than CCD-affected colonies.

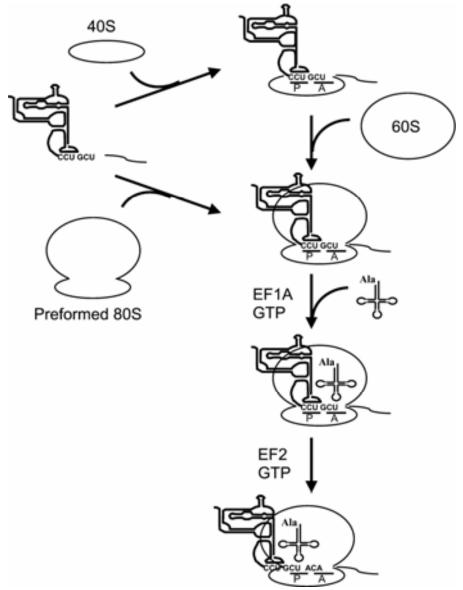
Conclusions/Significance: This is the first comprehensive survey of CCD-affected bee populations that suggests CCD involves an interaction between pathogens and other stress factors. We present evidence that this condition is contagious or the result of exposure to a common risk factor. Potentially important areas for future hypothesis-driven research, including the possible legacy effect of mite parasitism and the role of honey bee resistance to pesticides, are highlighted.

Citation: vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, et al. (2009) Colony Collapse Disorder: A Descriptive Study. PLoS ONE 4(8): e6481. doi:10.1371/journal.pone.0006481



The 5' non-translated region (NTR) of picornaviruses and picorna-like viruses (Dicistroviridae) contains a functional domain called the internal ribosome entry site (IRES) (members of the Dicistroviridae possess two open reading frames translated by two IRESes). The IRES is used by a virus to hijack the translation machinery in the host so the host translates viral mRNA instead of its own mRNA

Figure 1 Model illustrating the recruitment of ribosomal subunits by the diverse IRES elements located in the insect Dicistroviridae viruses



**Biochemical Society Transactions** 

www.biochemsoctrans.org 33, 1479-1482

Biochem. Soc. Trans. (2005)

#### Multiple viral infections are increasingly common...

ELSEVIER

Journal of Invertebrate Pathology 87 (2004) 84-93

www.elsevier.com/locate/yjipa

# Multiple virus infections in the honey bee and genome divergence of honey bee viruses

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Received 17 May 2004; accepted 19 July 2004 Available online 28 September 2004

#### Abstract

Using uniplex RT-PCR we screened honey bee colonies for the presence of several bee viruses, including black queen cell virus (BQCV), deformed wing virus (DWV), Kashmir bee virus (KBV), and sacbrood virus (SBV), and described the detection of mixed virus infections in bees from these colonies. We report for the first time that individual bees can harbor four viruses simultaneously. We also developed a multiplex RT-PCR assay for the simultaneous detection of multiple bee viruses. The feasibility and specificity of the multiplex RT-PCR assay suggests that this assay is an effective tool for simultaneous examination of mixed virus infections in bee colonies and would be useful for the diagnosis and surveillance of honey bee viral diseases in the field and laboratory. Phylogenetic analysis of putative helicase and RNA-dependent RNA polymerase (RdRp) encoded by viruses reveal that DWV and SBV fall into a same clade, whereas KBV and BQCV belong to a distinct lineage with other picorna-like viruses that infect plants, insects and vertebrates. Results from field surveys of these viruses indicate that mixed infections of BQCV, DWV, KBV, and SBV in the honey bee probably arise due to broad geographic distribution of viruses.



Home | Document

Apidologie 39 (2008) 310-314 DOI: 10.1051/apido:2008007

Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (Apis mellifera) in Denmark

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Received 31 July 2007 - Revised 13 December 2007 - Accepted 13 December 2007 - Published online 10 April 2008

Abstract - Samples of adult honey bees from apiaries with unusually high winter mortality and brood from hives with symptoms of disease were tested for presence of acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), Kashmir bee virus (KBV) and sacbrood virus (SBV) by RT-PCR. All six viruses were detected, but the frequencies varied significantly: SBV was detected in 78 apiaries, DWV in 55, ABPV in 11, CBPV in 4, BQCV in 1 and KBV in 1. This is the first record of KBV in Denmark. A large majority of the bee samples were infected with one or more viruses. Single, dual and triple infections were observed. Nucleotide sequences of the PCR products from each virus were determined and found to be 98-99% identical to GenBank accessions except CBPV, which was only 88-90% identical to known CBPV sequences.



...all over the world...

...probably as a result of globalization of trade...

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Journal of Invertebrate Pathology 98 (2008) 235-238

www.elsevier.com/locate/yjipa

#### Short Communication

#### Prevalence of pathogenic bee viruses in Hungarian apiaries: Situation before joining the European Union

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Received 9 August 2007; accepted 12 November 2007 Available online 19 November 2007

#### Abstract

A survey on the occurrence of six honeybee-pathogenic viruses was carried out using one-step RT-PCR assays. Samples were collected between 1999 and 2004 in 52 Hungarian apiaries located in different regions of the country. The results of the assays on samples of adult honeybees and Varroa destructor mites were compared to similar surveys from France and Austria. The study demonstrates geographical differences in the prevalence of honeybee viruses between Hungary and the older EU member states. The results could serve as a basis for monitoring further changes in the distribution of honeybee viruses in Europe.



Although the microarray analysis doesn't directly identify a cause, it provides tools for genome-enabled diagnosis so that beekeepers will have earlier opportunities to take action

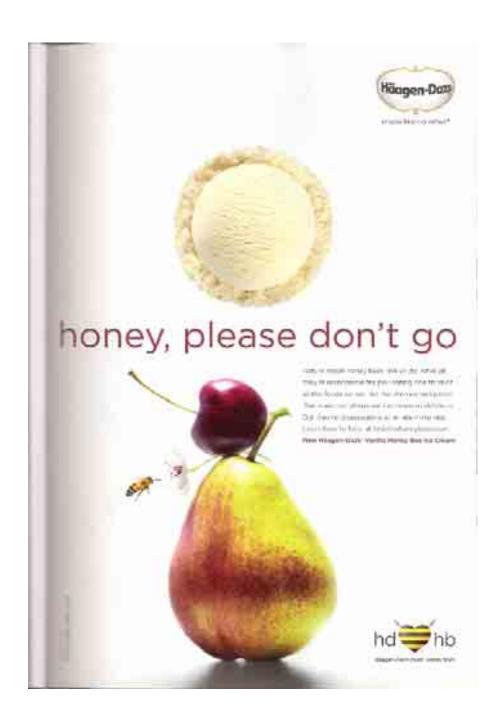
# Curing CCD won't

- protect bees against future pests or pathogens (increasingly likely with globalization of trade)
- provide "crop insurance" in the form of alternative pollinators
- maintain wild populations of pollinators to insure the vitality of both managed and natural plant communities



It's unlikely that honey bees will go extinct (there are close to two dozen races across the globe), but the globe), but the beekeeping industry in the U.S. might not survive; prospects for survival of wild pollinators are impossible to assess without baseline data

Unlike sunshine, pollination is not an inexhaustible resource



### Thanks!

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Amy Toth
Art Zangerl
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