

# Scientific papers, mostly with abstracts, relating to near-natural beekeeping

Compiled by David Heaf and revised May 2020  
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Since this compilation was created Peter Neumann and Tjeerd Blacquière have published a paper in *Evolutionary Applications* which succinctly covers many of the issues raised here. The authors point out that although several factors have been put forward as causes of the decline in honey bee health and colony numbers, the role of beekeeping practices in this decline has been largely ignored. Bibliographic data of this paper and a full abstract are presented at the head of the list of papers below.

Many of the papers mentioned are available through open access on the Internet, especially those published in PLoS ONE. If a desired paper is not on the journal publisher's own web site it is sometimes downloadable from the web site of one of the authors, usually within a university department web site. Another useful source of downloadable publications is researchgate.net. Failing all else, most authors of papers are pleased to send a PDF copy to people who are interested in their work. Commonly, the corresponding author's email address is on the web page for the article on the journal publisher's web site. Failing that, a contact e-address usually can be found on the web site of the author or of one of the co-authors. These are traceable through the author affiliations given on the web page for the article on the journal publisher's web site.

There is no topic below that is devoted specifically to natural comb. However, some of the topics covered are tangentially relevant to natural comb: 1. feral nests have about 17% drone cells whereas only worker foundation is commonly used; 2. worker cell sizes in natural comb range from about 4.6 to 5.6 mm whereas foundation has a uniform cell size; 3. recycled beeswax in foundation contains pesticides; 4. natural comb provides a free hanging medium for vibration communication whereas wired foundation in frames dampens the vibrations. 5. Natural comb is usually fixed in its full width at the top of the cavity and part way down at the edges, whereas framed combs have gaps across the top and down the sides. This undermines *Nestduftwärmehindung* (retention of nest scent and heat). 6. Natural comb has a lower ecological footprint than comb built on plastic or wax foundation. Suggestions for additional material on natural comb would be welcomed.

## Topics covered:

- Colony density and the lesson from ferals
- Bees can co-adapt with Varroa, and/or develop tolerance to Varroa and its vectored viruses
- Pesticide residues including acaricides (miticides) in brood comb, beeswax, foundation etc.
- Toxicity of acaricides used against Varroa
- Feral nest structure and criteria for nest selection by swarms
- Advantage of intracolony genetic diversity obtained through plentiful drone populations enabling adequate multiple mating and consequent fitness
- Role of drones in nest thermoregulation
- Importance of species-specific behaviours, e.g. 'social immunity', in disease resistance
- Hive magnetic materials and ambient electromagnetic radiation
- Pheromone integrity and distribution
- Pollen nutrition and diversity; forage quality and abundance
- Regulation of temperature and humidity
- Suppression of reproductive swarming and associated beekeeping practices may adversely affect bee health
- Foul brood incidence is higher in managed colonies compared with ferals
- Comb with small cell foundation is ineffective against Varroa
- Mesh floors – pros and cons as regards Varroa elimination
- Queen production, mating, artificial insemination and physiology
- Queen breeding, genetic diversity and local adaptation

- Beneficial bacteria versus pathogens
- Beekeeper applied antibiotics and antibiotic resistance in gut microbiota
- Presence of organic acids in the hive atmosphere (cf. Johann Thür's *Nestduftwärmehindung* – retention of nest scent and heat)
- Swarm lures and bait hives for swarm management where swarm suppression is not practised
- Effect of feeding sugar or not feeding honey; role of self-medication
- Comb as a vibration communication medium
- Beekeeping contributes to rather than detracts from conservation biology
- International trade in honey bees spreads pests and pathogens
- Non-invasive colony monitoring
- Health impacts of migratory beekeeping
- Antimicrobial properties of brood comb (propolis)
- Comparison of extensive v. intensive apiculture

### "The Darwin cure for apiculture? Natural selection and managed honey bee health"

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Recent major losses of managed honey bee, *Apis mellifera*, colonies at a global scale have resulted in a multitude of research efforts to identify the underlying mechanisms. Numerous factors acting singly and/or in combination have been identified, ranging from pathogens, over nutrition to pesticides. However, the role of apiculture in limiting natural selection has largely been ignored. This is unfortunate, because honey bees are more exposed to environmental stressors compared to other livestock and management can severely compromise bee health. Here, we briefly review apicultural factors that influence bee health and focus on those most likely interfering with natural selection, which offers a broad range of evolutionary applications for field practice. Despite intense breeding over centuries, natural selection appears to be much more relevant for the health of managed *A. mellifera* colonies than previously thought. We conclude that sustainable solutions for the apicultural sector can only be achieved by taking advantage of natural selection and not by attempting to limit it.

Open access: <http://onlinelibrary.wiley.com/doi/10.1111/eva.12448/abstract>

**COMMENT:** The above paper cites the following review in which again natural selection is mentioned as a possible cause of the relatively good state of health of honey bees in Africa.

Honeybee health in Africa—a review

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Received 30 April 2015—Revised 9 October 2015—Accepted 26 October 2015—Published online 30 November 2015

**Abstract**—Honeybee (*Apis mellifera* L.) pathogens and parasites and the negative effects thereof on honeybee populations remain an issue of public concern and the subject of active research. Africa with its high genetic diversity of honeybee sub-species and large wild population is also exposed to various factors responsible for colony losses in other parts of the world. Apart from the current American foulbrood epidemic in the Western Cape of South Africa, no large-scale colony losses have been reported elsewhere on the continent. We discuss the presence of pathogens, parasites, pests and predators of African honeybees as well as the threats they face in relation to habitat changes arising from the impact of increased human populations. In addition, we discuss current efforts aimed at protecting and promoting the health of African honeybees.

Apidologie Review article \*INRA, DIB and Springer-Verlag France, 2015, DOI:10.1007/s13592-015-0406-

## Why Gentle Honeybee Hives Are Less Resilient

Burke, Katie L.

American Scientist; Research Triangle Park

Vol. 108, Iss. 2, (Mar/Apr 2020): 69-70.

ARTICLE PREVIEW Animal behaviorist Clare Rittschof of the University of Kentucky has been studying honeybee aggression for more than 10 years, so it might come as no surprise that she's lost count of the number of times she's been stung. But she used to keep count. "I got 88 honeybee stings just in one summer," she recalls.

That's because it can actually be better for her and the bees if she does occasionally get stung. "I don't really go for 100 percent no sting," she says. "I don't wear gloves or anything on my hands, because I find it easier to handle the bees. Also, if you get a few stings here and there, it can help maintain a lower reaction level." Safety comes first in her lab, of course, and sting management is a part of it. "You're going into the nest, into the lion's den. You don't necessarily observe from a safe distance," she says. "It takes a long time to train students." But experiencing honeybee aggression is not just a byproduct of her research--it's a focus.

Studies have shown that low-aggression hives have higher mortality rates than high-aggression ones.

Scientists across many disciplines are trying to figure out why honeybee colonies are dying at historically high rates. Many stressors--such as infections, pesticides, and a lack of nutritious food--contribute to this mortality. Understanding those synergistic effects of stress, and what they have to do with low aggression, could help curb colony losses. In a recent study published in *BMC Genomics*, Rittschof identified the molecular reasons that low-aggression bee hives are more susceptible to stressors. (In this case, Rittschof is not talking about "killer bees," which are especially aggressive hybrids between the European honeybee and the East African lowland honeybee.)

## Colony density and the lesson from ferals

Contribution of European forests to safeguard wild honeybee populations

Fabrice Requier, Yoan Paillet, Fabien Laroche, Benjamin Rutschmann, Jie Zhang, Fabio Lombardi,

Miroslav Svoboda., Ingolf Steffan-Dewenter

<https://conbio.onlinelibrary.wiley.com/doi/pdf/10.1111/conl.12693>

ABSTRACT Recent studies reveal the use of tree cavities by wild honeybee colonies in European forests.

This highlights the conservation potential of forests for a highly threatened component of the native entomofauna in Europe, but currently no estimate of potential wild honeybee population sizes exists. Here, we analyzed the tree cavity densities of 106 forest areas across Europe and inferred an expected population size of wild honeybees. Both forest and management types affected the density of tree cavities. Accordingly, we estimated that more than 80,000 wild honeybee colonies could be sustained in European forests. As expected, potential conservation hotspots were identified in unmanaged forests, and, surprisingly, also in other large forest areas across Europe. Our results contribute to the EU policy strategy to halt pollinator declines and reveal the potential of forest areas for the conservation of so far neglected wild honeybee populations in Europe.

The natural control of the tracheal mite of honey bees

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Received 3 January 2001; accepted in revised form 23 August 2001

Bailey, L. & Perry, J. N. (2001) The natural control of the tracheal mite of honey bees. *Exp. Appl. Acarol.* **25** 745–749.

Key words: *Acarapis woodi*, Honey bee, Isle of Wight Disease, Natural control, Paralysis virus

Abstract. The natural control of the tracheal mite, *Acarapis woodi*, of the honey bee depends greatly on good foraging opportunities for bee colonies and on limited competition between colonies. The severe, widely-publicised disease of bees, referred to as 'the Isle of Wight disease' in Britain early in the last century when colonies were more numerous than subsequently, was wrongly attributed to the mite, which causes no overt symptoms. The disease was almost certainly caused by bee paralysis virus, which does cause the symptoms and is independent of the mite, and which is similarly associated with large

densities of bee colonies.

Experimental and Applied Acarology 25: 745–749, 2001.

Apidologie 38 (2007) 19-29

DOI: 10.1051/apido:2006055

Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States

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(Received 21 January 2006 - Revised 23 April 2006 - Accepted 23 April 2006 - Published online 29 November 2006)

Abstract - Feral colonies of European honey bees living in the Arnot Forest, a 1651-ha research preserve in New York State, were studied over a three-year period, 2002 to 2005. This population of colonies was previously censused in 1978. A census in 2002 revealed as many colonies as before, even though *Varroa destructor* was introduced to North America in the intervening years. Most colonies located in fall 2002 were still alive in fall 2005. The Arnot Forest colonies proved to be infested with *V. destructor*, but their mite populations did not surge to high levels in late summer. To see if Arnot Forest bees can suppress the reproduction rate of mites, colonies of Arnot Forest bees and New World Carniolan bees were inoculated with mites from an apiary and the growth patterns of their mite populations were compared. No difference was found between the two colony types. Evidently, the stable bee-mite relationship in the Arnot Forest reflects adaptations for parasite (mite) avirulence, not host (bee) resistance.

**COMMENT: In the above study, the mean distance between colonies was 1000 metres approx.**

Spatial and Temporal Distribution and Nest Site Characteristics of Feral Honey Bee (Hymenoptera: Apidae) Colonies in a Coastal Prairie Landscape

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Environ. Entomol. 34(3): 610D618 (2005)

ABSTRACT We evaluated the distribution and abundance of feral honey bee, *Apis mellifera* L., colonies in a coastal prairie landscape by examining nest site characteristics, population trends, and spatial and temporal patterns in cavity use. The colony densities of up to 12.5 colonies per km<sup>2</sup> were the highest reported in the literature for an area including both suitable and unsuitable patches of nesting habitat. The measured cavity attributes were similar to those reported from other areas. The time occupied and turnover indices provided useful information about cavity quality, although none of the measured cavity attributes were correlated with these indices. Unmeasurable cavity characteristics, such as cavity volume, may provide a better estimate of cavity quality. Spatial patterns existed in cavity use by the feral colonies, with the colonies showing an aggregated pattern of distribution throughout the study. Colony aggregations probably resulted from the distribution of resources, especially cavities. Two years after the arrival of Africanized honey bees, cavities used by Africanized and European colonies were aggregated in distribution. During what seemed to be a transition period, both Africanized and European colonies were randomly distributed. After that time, European colonies remained randomly distributed, whereas Africanized colonies were aggregated. Therefore, the invasion of Africanized honey bees seemed to fragment the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

Comparing honeybee densities:

European semi-natural habitats versus African deserts

Jaffé R, Shaibi T, Dietemann V, Kraus FB, Crewe R, Moritz RFA (2007): Comparing honeybee densities:

European semi-natural habitats versus African deserts (poster). Sustainable Neighbourhood - from Lisbon to Leipzig through Research (L2L): May 8th - 10th, Leipzig, Germany.

Jaffé, R., Shaibi, T., Dietemann, V., Kraus, F.B., Crewe, R. & Moritz, R. F. A. Comparing honeybee densities: European semi-natural habitats versus African deserts. (2007) (poster). Sustainable Neighbourhood - from Lisbon to Leipzig through Research (L2L): May 8th - 10th, Leipzig, Germany.

## Extended Abstract

In the light of a possible global pollinator decline (Allen-Wardel et al., 1998; Ghazoul, 2005) it is important to assess the current status of key pollinators, particularly in areas where important natural habitat losses have been taking place. Kept by apiculturists all over the world both for honey production and pollination services, the honeybee *Apis mellifera* is a pollinator of paramount importance for the maintenance of food supply systems. In this study we employed genetic markers to quantify the density of honeybee colonies in different semi-natural regions of Europe. Using a set of tightly linked microsatellite markers (Lattorff et al., in prep), we genotyped drones and worker offspring from mated queens (Kraus et al., 2005). Since honeybee queens mate with many different haploid males, we were able to get a robust sample of male haplotypes from each population. Colony density was then estimated based on the reconstructed number of drone contributing queens and the flight distances of queens and drones. In order to compare European honeybee densities with those of natural ecosystems under harsh conditions, we analyzed samples from the African deserts Sahara and Kalahari, following the same procedures. The density of honeybee colonies in European populations (between 5 and 6 col/sq km) was found to be significantly lower than that of African deserts (between 10 and 15 col/sq km), while the expected heterozygosity ( $H_e$ ) and the allele richness ( $A$ ) in both regions showed similar ranges ( $H_e$ : 0.68- 0.84 and  $A$ : 5-12). Hence, here we show that in spite of intense beekeeping, European honeybee populations exhibit lower densities and similar genetic diversity as populations sampled from African deserts. Although climatic factors and racial differences are likely to affect wild honeybee densities (Seeley, 1985), our results suggest that the apicultural activities in Europe are unlikely to compensate for the loss of habitats suitable for wild honeybees, due to agriculture, forestry and other utilization of land.

## ALSO

Jaffé R, Shaibi T, Dietemann V, Kraus FB, Crewe R, Moritz RFA (2007): Comparing honeybee densities: European versus African semi-natural and natural habitats. 54. Jahrestagung der Arbeitsgemeinschaft der Institut für Bienenforschung e.V.: March 27th - 29th, Würzburg, Germany.

Jaffé R, Shaibi T, Dietemann V, Kraus FB, Crewe R, Moritz RFA (2006): Comparing wild honeybee densities: African deserts vs. European semi-natural habitats. 2nd European Conference of Apidology: September 10th – 14th, Prague, Czech Republic.

## PSYCHE

Vol. 84 September-December, 1977 No. 3-4

### DISPERSAL BEHAVIOR OF HONEY BEE SWARMS\*

BY THOMAS D. SEELEY AND ROGER A. MORSE

Seeley, T. D. & Morse, R. A. (1977) Dispersal behaviour of honey bee swarms. *Psyche* **84**(3-4) 199-209. Museum of Comparative Zoology

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Insofar as normal honey bee behavior was observed in these studies with artificial swarms, our results indicate that swarms frequently move at least 300 meters from their parent colony to a new nest site, but that they do not prefer nest sites far from their parent colonies. Instead, swarms may prefer a nest site which is near the parent colony. Therefore the spacing of suitable nest sites appears to be a major determinant of the spacing of feral honey bee colonies, and behaviors promoting colony spacing to reduce foraging competition may not exist in the European races of honey bees.

The density of feral honey bee (*Apis mellifera*) colonies in South East Australia is greater in undisturbed than in disturbed habitats

Eloise M. HINSON, Michael DUNCAN, Julianne LIM, Jonathan ARUNDEL, Benjamin P. OLDROYD

Abstract— *Apis mellifera* is an important pollinator but is sometimes associated with adverse effects on natural ecosystems. We surveyed pairs of disturbed and undisturbed sites across three biomes in South East Australia. We used pheromone lures to trap drones, genotyped the drones to infer the number of colonies within flight range and then estimated colony densities using synthetic sampling distributions. Estimated colony densities ranged from 0.1 to 1.5 colonies  $\text{km}^{-2}$  and significantly lower in agricultural land relative to national parks. We suggest that colony density may be lower in disturbed than 'natural' areas due to the

reduced availability of nest sites and possibly pesticide usage. Because the number of colonies recommended for adequate pollination is 100–1000 colonies km<sup>-2</sup>, there are insufficient bees to provide optimal crop pollination in the areas we surveyed.

Apidologie Original article

\*INRA, DIB and Springer-Verlag France, 2015

DOI:10.1007/s13592-014-0334-x

Remarkable uniformity in the densities of feral honey bee *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) colonies in South Eastern Australia

Jonathan Arundell\*, Peter R Oxley, Alen Faiz, John Crawford, Stephan Winter and Benjamin P Oldroyd  
Austral Entomology

Volume 53, Issue 3, pages 328–336, August 2014

Abstract

It is often assumed that the density of feral honey bee colonies in Australia is sufficient to provide adequate pollination services to the many agricultural crops that require pollination. In contrast, there is concern that the density of feral colonies is sufficiently high to have inimical effects on Australian biota. For both these reasons, it is desirable to have robust estimates of the density of feral honey bee colonies in Australian landscapes. In this study, we mated four to five queens with wild drones at disturbed and undisturbed sites in three of the major ecosystems in Victoria Australia and examined the paternities of worker offspring to estimate the density of feral colonies within mating range of the test queens. We show that the density of feral colonies differs little with land use (cleared or uncleared) and is similar across the state. Our data suggest that the density of feral colonies is probably insufficient to provide adequate pollination of agricultural crops and that neither land use nor local climate variation is a major factor determining density. Finally, our data suggest that the mating range sampled by test queens is significantly greater than previously assumed.

Apidologie (1999) **26**, 119-130

Colony aggregations in *Apis mellifera* L

B Oldroyd A Smolenski S Lawler A Estoup R Crozier

Summary

Natural aggregations of *A. mellifera* L have not been reported. However, in the related species *A. dorsata*, aggregations of colonies are common. A survey of the spatial distribution of feral *mellifera* colonies showed that they too can be markedly clumped, with up to 10 colonies/ha. For these heavily clumped colonies, we inferred queen genotype from worker samples for: 1) malate dehydrogenase; 2) a mitochondrial DNA polymorphism; and 3) a microsatellite locus. The aggregation examined was composed of colonies headed by potentially related (ie parent/offspring or sister) queens, and unrelated colonies. Thus, it is likely that existing colonies attract swarms and that swarms may not always travel far from the natal nest in an environment that is replete with nesting sites.

Apidologie, 46 (6), 716-727. 2015

Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*

Thomas D. Seeley, Michael L. Smith

Abstract

When humans switched from hunting honeybee colonies living scattered in the wild to keeping them in hives crowded in apiaries, they may have greatly increased disease transmission between colonies. The effects of clustering colonies were studied. Two groups of 12 colonies, with hives crowded or dispersed, were established in a common environment and left untreated for mites. Drones made many homing errors in the crowded group, but not in the dispersed group. In early summer, in both groups, the colonies that did not swarm developed high mite counts, but the colonies that swarmed maintained low mite counts. In late summer, in the crowded group but not in the dispersed group, the colonies that swarmed also developed high mite counts. All colonies with high mite counts in late summer died over winter; all colonies with low mite counts in late summer survived over winter. Evidently, swarming can reduce a colony's mite load, but when colonies are crowded in apiaries, this mite-load reduction is erased as mites are spread through drifting and robbing.

## Urbanization Increases Pathogen Pressure on Feral and Managed Honey Bees

Elsa Youngsteadt, R. Holden Appler, Margarita M. López-Uribe, David R. Tarpy, Steven D. Frank

Given the role of infectious disease in global pollinator decline, there is a need to understand factors that shape pathogen susceptibility and transmission in bees. Here we ask how urbanization affects the immune response and pathogen load of feral and managed colonies of honey bees (*Apis mellifera* Linnaeus), the predominant economically important pollinator worldwide. Using quantitative real-time PCR, we measured expression of 4 immune genes and relative abundance of 10 honey bee pathogens. We also measured worker survival in a laboratory bioassay. We found that pathogen pressure on honey bees increased with urbanization and management, and the probability of worker survival declined 3-fold along our urbanization gradient. The effect of management on pathogens appears to be mediated by immunity, with feral bees expressing immune genes at nearly twice the levels of managed bees following an immune challenge. The effect of urbanization, however, was not linked with immunity; instead, urbanization may favor viability and transmission of some disease agents. Feral colonies, with lower disease burdens and stronger immune responses, may illuminate ways to improve honey bee management. **The previously unexamined effects of urbanization on honey-bee disease are concerning, suggesting that urban areas may favor problematic diseases of pollinators.**

**COMMENT: The following paper is related to the previous one.**

## Within-Colony Variation in the Immunocompetency of Managed and Feral Honey Bees (*Apis mellifera* L.) in Different Urban Landscapes

R. Holden Appler, Steven D. Frank and David R. Tarpy

Insects 2015, 6, 912-925; doi:10.3390/insects6040912

Abstract: Urbanization has the potential to dramatically affect insect populations worldwide, although its effects on pollinator populations are just beginning to be understood. We compared the immunocompetency of honey bees sampled from feral (wild-living) and managed (beekeeper-owned) honey bee colonies. We sampled foragers from feral and managed colonies in rural, suburban, and urban landscapes in and around Raleigh, NC, USA. We then analyzed adult workers using two standard bioassays for insect immune function (encapsulation response and phenoloxidase activity). We found that there was far more variation within colonies for encapsulation response or phenoloxidase activity than among rural to urban landscapes, and we did not observe any significant difference in immune response between feral and managed bees. These findings suggest that social pollinators, like honey bees, may be sufficiently robust or variable in their immune responses to obscure any subtle effects of urbanization. Additional studies of immune physiology and disease ecology of social and solitary bees in urban, suburban, and natural ecosystems will provide insights into the relative effects of changing urban environments on several important factors that influence pollinator productivity and health.

Forfert, Nadège, Natsopoulou, Myrsini E., Paxton, Robert J., Moritz, Robin F.A.,

Viral prevalence increases with regional colony abundance in honey bee drones (*Apis mellifera* L.), (2016), doi:10.1016/j.meegid.2016.07.017

Transmission among colonies is a central feature for the epidemiology of honey bee pathogens. High colony abundance may promote transmission among colonies independently of apiary layout, making colony abundance a potentially important parameter determining pathogen prevalence in populations of honey bees. To test this idea, we sampled male honey bees (drones) from seven distinct drone congregation areas (DCA), and used their genotypes to estimate colony abundance at each site. A multiplex ligation dependent probe amplification assay (MLPA) was used to assess the prevalence of ten viruses, using five common viral targets, in individual drones. There was a significant positive association between colony abundance and number of viral infections. This result highlights the potential importance of high colony abundance for pathogen prevalence, possibly because high population density facilitates pathogen transmission. Pathogen prevalence in drones collected from DCAs may be a useful means of estimating the disease status of a population of honey bees during the mating season, especially for localities with a large number of wild or feral colonies.

Invasion of *Varroa destructor* mites into mite-free honey bee colonies under the controlled conditions of

a military training area

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Received 27 April 2010, accepted subject to revision 30 November 2010, accepted for publication 1 March 2011.

### Summary

The honey bee mite *Varroa destructor* can be spread between colonies by vertical transmission, particularly when heavily infested colonies are robbed by foraging bees from neighbouring hives. We quantified the invasion of *V. destructor* into mite free colonies on a military training area not accessible to other beekeepers. Ten “mite receiver colonies” continuously treated against *V. destructor* were placed at distances of one to 1.5 km from four heavily infested “mite donor colonies”. Over a two month period from August to October, the population of bees, brood, and *V. destructor* in the donor colonies were estimated at three week intervals and the invasion of *V. destructor* into the receiver colonies was recorded every 7-12 days. During the experimental period, between 85 and 444 mites per colony were introduced into the receiver colonies. There were no significant differences in the invasion rates in relation to the distance between donor and receiver colonies. In total, 2,029 mites were found in the 10 receiver colonies, but these only correspond to 2.5% of the total mite population in the donor colonies at the start of the experiment. This means that the major part of the initial *V. destructor* population died together with the collapsed host colonies. Under natural conditions, a more benign behaviour should therefore be an adaptive strategy for *V. destructor*. From a practical perspective we could show that highly infested honey bee colonies present a substantial risk to already treated colonies up to distances of 1.5 km away.

Journal of Apicultural Research 50(2): 138-144 (2011) DOI 10.3896/IBRA.1.50.2.05

### Autumn Invasion Rates of *Varroa destructor* (Mesostigmata: Varroidae) Into Honey Bee (Hymenoptera: Apidae) Colonies and the Resulting Increase in Mite Populations

Source: Journal of Economic Entomology, 107(2):508-515. 2014. DOI: <http://dx.doi.org/10.1603/EC13381>

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**ABSTRACT** The honey bee parasite *Varroa destructor* Anderson & Trueman can disperse and invade honey bee colonies by attaching to “drifting” and “robbing” honey bees that move into nonnatal colonies. We quantified the weekly invasion rates and the subsequent mite population growth from the end of July to November 2011 in 28 honey bee colonies kept in two apiaries that had high (HBD) and low (LBD) densities of neighboring colonies. At each apiary, half (seven) of the colonies were continuously treated with acaricides to kill all *Varroa* mites and thereby determine the invasion rates. The other group of colonies was only treated before the beginning of the experiment and then left untreated to record *Varroa* population growth until a final treatment in November. The numbers of bees and brood cells of all colonies were estimated according to the Liebefeld evaluation method. The invasion rates varied among individual colonies but revealed highly significant differences between the study sites. The average invasion rate per colony over the entire 3.5-mo period ranged from 266 to 1,171 mites at the HBD site compared with only 72 to 248 mites at the LBD apiary. In the untreated colonies, the *Varroa* population reached an average final infestation in November of 2,082 mites per colony (HBD) and 340 mites per colony (LBD). All colonies survived the winter; however, the higher infested colonies lost about three times more bees compared with the lower infested colonies. Therefore, mite invasion and late-year population growth must be considered more carefully for future treatment concepts in temperate regions.

### Distance between honey bee *Apis mellifera* colonies regulates populations of *Varroa destructor* at a landscape scale

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Received 16 October 2015–Revised 27 January 2016–Accepted 23 March 2016

**Abstract**– Inter-colony distance of *Apis mellifera* significantly affects colony numbers of the parasitic mite *Varroa destructor*. We set up 15 apiaries, each consisting of two colonies. Each apiary pair was assigned an inter-colony distance of 0, 10, or 100 m. Colonies were rendered nearly mite-free, then one colony in each pair was seeded with 300 female mites (mite-donor colony), while the other remained uninoculated



(mite-recipient colony). After 4 months of monitoring, a whole-model analysis showed that apiaries in which colonies were spaced 100 m apart contained lower average mite numbers than 0 or 10 m apiaries. There were interactions among colony type, distance, and sampling date; however, when there were significant differences, mite numbers were always lower in 100 m apiaries than 10 m apiaries. These findings pose the possibility that *Varroa* populations are resource regulated at a landscape scale: near-neighbor colonies constitute reproductive resource for mites in the form of additional bee brood. *Apidologie Online* 2 May 2016. This article is published with open access at Springerlink.com DOI:10.1007/s13592-016-0443-9

Estimating the Density of Honeybee Colonies across Their Natural Range to Fill the Gap in Pollinator Decline Censuses

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**Abstract:** Although pollinator declines are a global biodiversity threat, the demography of the western honeybee (*Apis mellifera*) has not been considered by conservationists because it is biased by the activity of beekeepers. To fill this gap in pollinator decline censuses and to provide a broad picture of the current status of honeybees across their natural range, we used microsatellite genetic markers to estimate colony densities and genetic diversity at different locations in Europe, Africa, and central Asia that had different patterns of land use. Genetic diversity and colony densities were highest in South Africa and lowest in Northern Europe and were correlated with mean annual temperature. Confounding factors not related to climate, however, are also likely to influence genetic diversity and colony densities in honeybee populations. Land use showed a significantly negative influence over genetic diversity and the density of honeybee colonies over all sampling locations. In Europe honeybees sampled in nature reserves had genetic diversity and colony densities similar to those sampled in agricultural landscapes, which suggests that the former are not wild but may have come from managed hives. Other results also support this idea: putative wild bees were rare in our European samples, and the mean estimated density of honeybee colonies on the continent closely resembled the reported mean number of managed hives. Current densities of European honeybee populations are in the same range as those found in the adverse climatic conditions of the Kalahari and Saharan deserts, which suggests that beekeeping activities do not compensate for the loss of wild colonies. **Our findings highlight the importance of reconsidering the conservation status of honeybees in Europe and of regarding beekeeping not only as a profitable business for producing honey, but also as an essential component of biodiversity conservation.**

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**The following paper is an attempt to estimate feral colony densities in Poland.**

Rural avenues as a refuge for feral honey bee population

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*J Insect Conserv* (2013) 17:465–472. DOI 10.1007/s10841-012-9528-6

**Abstract** Several honey bee (*Apis mellifera*) subspecies are in danger of local extinction because their feral population have almost completely disappeared. An important threat to the feral populations of bees is loss of habitat and loss of woodlands. In many places the only habitat suitable for honey bee nesting are rows of trees along roadsides. We studied a feral population of honey bees inhabiting avenues in northern Poland. We inspected 142 km of avenues and found 45 feral colonies. The estimated density of feral population inhabiting the avenues was 0.10 nest km<sup>-2</sup>. Honey bees preferred to build their nests in trees with a thick trunk and a somewhat weak state of health. There was no strong preference of bees to any species of trees. We stress the importance of protection of existing avenues and creating new ones. This can provide suitable habitat not only for honey bees but also for other endangered species.

Mite bombs or robber lures? The roles of drifting and robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors

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Thomas Dyer Seeley

June 21, 2019

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0218392>

When honey bee colonies collapse from high infestations of *Varroa* mites, neighboring colonies often experience surges in their mite populations. Collapsing colonies, often called “mite bombs”, seem to pass their mites to neighboring colonies. This can happen by mite-infested workers from the collapsing colonies drifting into the neighboring colonies, or by mite-free workers from the neighboring colonies robbing out the collapsing colonies, or both. To study inter-colony mite transmission, we positioned six nearly mite-free colonies of black-colored bees around a cluster of three mite-laden colonies of yellow-colored bees. We then monitored the movement of bees between the black-bee and yellow-bee colonies before, during, and after mite-induced collapse of the yellow-bee colonies. Throughout the experiment, we monitored each colony's mite level. We found that large numbers of mites spread to the black-bee colonies (in both nearby and distant hives) when the yellow-bee colonies collapsed from high mite infestations and became targets of robbing by the black-bee colonies. We conclude that “robber lures” is a better term than “mite bombs” for describing colonies that are succumbing to high mite loads and are exuding mites to neighboring colonies.

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0216286>

Reduced density and visually complex apiaries reduce parasite load and promote honey production and overwintering survival in honey bees

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<https://doi.org/10.1371/journal.pone.0216286>

Managed honey bee (*Apis mellifera*) colonies are kept at much greater densities than naturally occurring feral or wild colonies, which may have detrimental effects on colony health and survival, disease spread, and drifting behavior (bee movement between natal and non-natal colonies). We assessed the effects of a straightforward apiary management intervention (altering the density and visual appearance of colonies) on colony health. Specifically, we established three “high density / high drift” (“HD”) and three “low density / low drift” (“LD”) apiary configurations, each consisting of eight bee colonies. Hives in the HD apiary configuration were of the same color and placed 1m apart in a single linear array, while hives in the LD apiary configuration were placed 10m apart at different heights, facing outwards in a circle, and made visually distinctive with colors and symbols to reduce accidental drift between colonies. We investigated disease transmission and dynamics between the apiary configurations by clearing all colonies of the parasitic mite *Varroa destructor*, and subsequently inoculating two randomly-chosen colonies per apiary with controlled mite doses. We monitored the colonies for two years and found that the LD apiary configuration had significantly greater honey production and reduced overwinter mortality. Inoculation and apiary management intervention interacted to affect brood mite levels, with the highest levels in the inoculated colonies in the HD configuration. Finally, foragers were more than three times more likely to drift in the HD apiary configurations. Our results suggest that a relatively straightforward management change—placing colonies in low-density visually complex circles rather than high-density visually similar linear arrays—can provide meaningful benefits to the health and productivity of managed honey bee colonies.

## **Bees can co-adapt with *Varroa*, and/or develop tolerance to *Varroa* and its vectored viruses**

REVIEW: Natural *Varroa* amite-surviving *Apis mellifera* honeybee populations

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Received 18 April 2015—Revised 28 September 2015—Accepted 6 November 2015

Abstract—The *Varroa destructor* mite is the largest threat to apiculture worldwide and has been responsible for devastating losses of wild honeybee populations in Europe and North America. However, *Varroa* mite-

resistant populations of *A. mellifera* honeybees have been reported and documented around the world with a variety of explanations for their long-term survival with uncontrolled mite infestation. This review synthesizes the work on naturally occurring survival to *Varroa* mites and discusses what these honeybee populations can signify for apiculture.

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Martin, S.J., Hawkins, G.P., Brettell, L.E. et al.

*Varroa destructor* reproduction and cell re-capping in mite-resistant *Apis mellifera* populations. *Apidologie* (2019) doi:10.1007/s13592-019-00721-9

<https://link.springer.com/article/10.1007/s13592-019-00721-9>

**ABSTRACT** Globalization has facilitated the spread of emerging pests such as the *Varroa destructor* mite, resulting in the near global distribution of the pest. In South African and Brazilian honey bees, mite-resistant colonies appeared within a decade; in Europe, mite-resistant colonies are rare, but several of these exhibited high levels of “re-capping” behavior. We studied re-capping in *Varroa*-naïve (UK/Australia) and *Varroa*-resistant (South Africa and Brazil) populations and found very low and very high levels, respectively, with the resistant populations targeting mite-infested cells. Furthermore, 54% of artificially infested *A. m. capensis* worker cells were removed after 10 days and 83% of the remaining infested cells were re-capped. Such targeted re-capping of drone cells did not occur. We propose that cell opening is a fundamental trait in mite-resistant populations and that re-capping is an accurate proxy for this behavior.

Quote from the Discussion section: "Throughout Brazil and Africa, beekeeping pest management is minimal and so selective pressures for such traits have always been high. The constant management of a wide range of brood pest and pathogens throughout the Northern Hemisphere removes much of this selective pressure."

Assessing virulence of *Varroa destructor* mites from different honey bee management regimes

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*Apidologie* 2019

<https://link.springer.com/article/10.1007/s13592-019-00716-6>

**ABSTRACT** The mite *Varroa destructor* is an important honey bee parasite that causes substantial losses of honey bee colonies worldwide. Evolutionary theory suggests that the high densities at which honey bees are managed in large-scale beekeeping settings will likely select for mites with greater growth and virulence, thereby potentially explaining the major damage done by these mites. We tested this hypothesis by collecting mites from feral bee colonies, “lightly” managed colonies (those from small-scale sedentary operations), and “heavily” managed colonies (those from large-scale operations that move thousands of colonies across the US on a yearly basis). We established 8 apiaries, each consisting of 11 colonies from a standardized lightly managed bee background that were cleared of mites, and artificially infested each apiary with controlled numbers of mites from feral, lightly managed, or heavily managed bees or left uninoculated as negative control. We monitored the colonies for more than 2 years for mite levels, colony strength (adult bee population, brood coverage, and honey storage), and survival. As predicted by evolutionary theory, we found that colonies inoculated with mites from managed backgrounds had increased *V. destructor* mite levels relative to those with mites from feral colonies or negative controls. However, we did not see a difference between heavily and lightly managed colonies, and these higher mite burdens did not translate into greater virulence, as measured by reductions in colony strength and survival. Our results suggest that human management of honey bee colonies may favor the increased population growth rate of *V. destructor*, but that a range of potential confounders (including viral infections and genotype-by-genotype interactions) likely contribute to the relationship between mite reproduction and virulence.

De Jong D, Soares AEE. (1997) An isolated population of Italian bees that has survived *Varroa jacobsoni* infestation without treatment for over 12 years. *Am Bee J* 137: 742–745.

Twenty colonies of Italian bees, infested with *Varroa jacobsoni* were introduced to the Island of Fernando de Noronha, located near the equator off the coast of Brazil, in 1984. These colonies initiated a population that is isolated and protected from genetic contamination by 345 km of ocean. During the 13 years since that time, the colony numbers have increased to about 50 colonies in hives, and an undetermined number of wild colonies on the 26 square kilometers of this archipelago. This group of colonies is unique, as it is maintained

without any type of treatment, and yet there is no evidence of significant damage or colony mortality due to the varroa infestations. The mean infestation rates of the adult bees in the colonies, found to be about 26 mites per hundred bees in 1991, decreased to 19 in 1993, and 14 in 1996.

<http://www.imkervlaamseardennen.be/images/pdffolder/art-De-Jong-bee-survive-varroa.pdf>

Apidologie 38 (2007) 19-29

DOI: 10.1051/apido:2006055

Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States

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Abstract - Feral colonies of European honey bees living in the Arnot Forest, a 1651-ha research preserve in New York State, were studied over a three-year period, 2002 to 2005. This population of colonies was previously censused in 1978. A census in 2002 revealed as many colonies as before, even though *Varroa destructor* was introduced to North America in the intervening years. Most colonies located in fall 2002 were still alive in fall 2005. The Arnot Forest colonies proved to be infested with *V. destructor*, but their mite populations did not surge to high levels in late summer. To see if Arnot Forest bees can suppress the reproduction rate of mites, colonies of Arnot Forest bees and New World Carniolan bees were inoculated with mites from an apiary and the growth patterns of their mite populations were compared. No difference was found between the two colony types. Evidently, the stable bee-mite relationship in the Arnot Forest reflects adaptations for parasite (mite) avirulence, not host (bee) resistance.

**COMMENT: The following paper is a follow-up study on the same bee population that is the subject of the paper immediately above.**

A survivor population of wild colonies of European honeybees in the northeastern United States: investigating its genetic structure

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CARCIONE, Deborah A. DELANEY

Apidologie September 2015, Volume 46, Issue 5, pp 654-666

There is a widespread belief that wild colonies of European honeybees have been eradicated in Europe and North America, killed by viruses spread by the introduced ectoparasitic mite, *Varroa destructor*. In reality, however, several populations of wild colonies of honeybees in Europe and North America are persisting despite exposure to *Varroa*. To help understand how this is happening, we tested whether the bees in one of these populations of wild colonies—those living in and around the Arnot Forest (NY, USA)—are genetically distinct from the bees in the nearest managed colonies. We found that the Arnot Forest honeybees are genetically distinct from the honeybees in the two apiaries within 6 km of the forest. Evidently, the population of Arnot Forest honeybees is not supported by a heavy influx of swarms from the nearest managed colonies, which implies that it is self-sustaining. These results suggest that if a closed population of honeybee colonies is allowed to live naturally, it will develop a balanced relationship with its agents of disease. Indeed, it is likely to become well adapted to its local environment as a whole. We suggest four ways to modify beekeeping practices to help honeybees live in greater health.

Museum samples reveal rapid evolution by wild honey bees exposed to a novel parasite

Alexander S. Mikheyev, Mandy M.Y. Tin, Jatin Arora & Thomas D. Seeley

Nature Communications, 6 August 2015

DOI: 10.1038/ncomms8991 | [www.nature.com/naturecommunications](http://www.nature.com/naturecommunications)

Understanding genetic changes caused by novel pathogens and parasites can reveal mechanisms of adaptation and genetic robustness. Using whole-genome sequencing of museum and modern specimens, we describe the genomic changes in a wild population of honey bees in North America following the introduction of the ectoparasitic mite, *Varroa destructor*. Even though colony density in the study population is the same today as in the past, a major loss of haplotypic diversity occurred, indicative of a drastic mitochondrial

bottleneck, caused by massive colony mortality. In contrast, nuclear genetic diversity did not change, though hundreds of genes show signs of selection. The genetic diversity within each bee colony, particularly as a consequence of polyandry by queens, may enable preservation of genetic diversity even during population bottlenecks. These findings suggest that genetically diverse honey bee populations can recover from introduced diseases by evolving rapid tolerance, while maintaining much of the standing genetic variation.

Changes in Honey Bee (Hymenoptera: Apidae) Colony Swarming and Survival Pre- and Postarrival of *Varroa destructor*

(Mesostigmata: Varroidae) in Louisiana

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*Ann. Entomol. Soc. Am.* 101(5): 867Ð871 (2008)

Villa, J. D., Bustamante, D. M., Dunkley, J. P. & Escobar, L. A. (2008) Changes in Honey Bee (Hymenoptera: Apidae) Colony Swarming and Survival Pre- and Postarrival of *Varroa destructor*. *Ann. Entomol. Soc. Am.* **101**(5) 867-871.

**ABSTRACT** The impact of *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae) on colonies of *Apis mellifera* L. (Hymenoptera: Apidae) in southern Louisiana was evaluated by analyzing changes in swarming and longevity of colonies for 17 yr. Swarming rates were calculated from yearly captures of swarms in bait hives placed in Þve areas of Louisiana from 1991 to 2006. Colony longevity was monitored in 104 swarms established from 1990 to 2000 and followed until 2004. In the Þrst years, before *V. destructor*, average swarm capture rates ranged from 0.85 to 0.95 swarms per bait hive-year, and survival of colonies established from swarms averaged 14 mo. In years immediately after the arrival of *V. destructor* (1993Ð1996), swarming rates and colony longevity decreased to 0.36Ð0.60 swarms per bait hive-year and 10 mo, respectively. After 5 yr in the presence of *V. destructor*, both rates recovered to levels at least as high as those seen before varroa arrived; swarm capture rates were 0.75Ð1.04 swarms per bait hive-year and average longevity was 26 mo. Analysis of varroa infestations in three colonies established from swarms in 1997 showed the presence of varroa at oscillating densities for 5 to 8 yr. Possible causes for this apparent recovery are natural selection for resistance in honey bees, introgression of selected resistant genetic material or reduced virulence of the mites.

*Apidologie* 38 (2007) Available online at:

INRA/DIB-AGIB/ EDP Sciences, 2007 [www.apidologie.org](http://www.apidologie.org)

DOI: 10.1051/apido:2007040

Original article

Honey bee colonies that have survived *Varroa destructor*\*

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**Abstract** – We document the ability of a population of honey bee colonies to survive in France without *Varroa* suppression measures. We compared the mortality of collected *Varroa* surviving bee (VSB) stock with that of miticide-treated *Varroa*-susceptible colonies. *Varroa* infestation did not induce mortality in the VSB colonies. Some of the original colonies survived more than 11 years without treatment and the average survival of the experimental colonies was  $6.54 \pm 0.25$  years. Swarming was variable ( $41.50 \pm 9.94\%$ ) depending on the year. Honey production was significantly higher (1.7 times) in treated than in VSB colonies. For the first time since *Varroa* invaded France, our results provide evidence that untreated local honey bee colonies can survive the mite, which may be the basis for integrated *Varroa* management.

*Apidologie* 37 (2006) 564–570 564

INRA/DIB-AGIB/ EDP Sciences, 2006

DOI: 10.1051/apido:2006031

Original article

Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate\*  
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Received 7 November 2005 – Accepted 23 December 2005

Fries, I. Imdorf, A. & Rosenkranz, P. (2006) Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate *Apidologie* **37**, 564–570.

Abstract – An isolated honey bee population (N = 150) was established on the southern tip of Gotland, an island in the Baltic sea. After infestation with 36 to 89 *Varroa destructor* mites per colony, they were unmanaged and allowed to swarm. For over six years colonies were monitored for swarming, winter losses, infestation rate in the fall, and bee population size in the spring. Winter mortality rate decreased from 76% and 57% in the third and fourth years, to 13% and 19% in the fifth and sixth years. Swarming rates increased from zero the third field season to 57.1% and 36.4% in the last two years. The mite infestation on adult bees decreased during the last two years, from 0.47% in the third year to 0.19% and 0.22% respectively. Our data suggest that a host-parasite co-adaptation has occurred ensuring survival of both the host and the parasite. The mechanisms behind this co-adaptation require further study.

*Apidologie* 38 (2007) 525–533 Available online at:

INRA, EDP Sciences, 2007 [www.apidologie.org](http://www.apidologie.org)

DOI: 10.1051/apido:2007039

Original article

Possible host-parasite adaptations in honey bees infested by *Varroa destructor* mites\*

Ingemar Fries, Riccardo Bommarco

Fries, I. & Bommarco, R. (2007) Possible host-parasite adaptations in honey bees infested by *Varroa destructor* mites. *Apidologie* **38** 525–533.

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Received 19 March 2007 – Revised and Accepted 22 August 2007

Abstract – We investigated *Varroa destructor* mite population growth in a line of honey bee (*Apis mellifera*) colonies that have survived mite infestation for seven years without treatment (Bond colonies), and in a line of colonies that had been treated to control the mites (Controls). We investigated if the source of mites affected mite population growth. The results showed that the overall mite population growth rate was reduced by 82% in Bond colonies compared to Control colonies, irrespective of the mite source (mites from Bond or Control colonies). Two traits may partly explain the difference seen in mite population growth. First, Bond colonies produced less worker and drone brood compared to Control colonies. Second, Control colonies had a larger proportion of the mites in the sealed brood compared to Bond colonies. Reduced brood production and traits leading to differences in mite distribution could be interpreted as adaptive responses to mite pressure, although a causal relationship was not demonstrated.

A selective sweep in a *Varroa destructor* resistant honeybee (*Apis mellifera*) population

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abstract

The mite *Varroa destructor* is one of the most dangerous parasites of the Western honeybee (*Apis mellifera*) causing enormous colony losses worldwide. Various chemical treatments for the control of the *Varroa* mite are currently in use, which, however, lead to residues in bee products and often to resistance in

mites. This facilitated the exploration of alternative treatment methods and breeding for mite resistant honeybees has been in focus for breeders in many parts of the world with variable results.

Another approach has been applied to a honeybee population on Gotland (Sweden) that was exposed to natural selection and survived *Varroa*-infestation for more than 10 years without treatment. Eventually this population became resistant to the parasite by suppressing the reproduction of the mite. A previous QTL mapping study had identified a region on chromosome 7 with major loci contributing to the mite resistance. Here, a microsatellite scan of the significant candidate QTL regions was used to investigate potential footprints of selection in the original population by comparing the study population on Gotland before (2000) and after selection (2007). Genetic drift had caused an extreme loss of genetic diversity in the 2007 population for all genetic markers tested. In addition to this overall reduction of heterozygosity, two loci on chromosome 7 showed an even stronger and significant reduction in diversity than expected from genetic drift alone. Within the selective sweep eleven genes are annotated, one of them being a putative candidate to interfere with reduced mite reproduction. A glucose–methanol–choline oxidoreductase (GMCOX18) might be involved in changing volatiles emitted by bee larvae that might be essential to trigger oogenesis in *Varroa*.

Infection, Genetics and Evolution 31 (2015) 169–176

Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation

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Abstract– A population of European honey bees (*Apis mellifera*) surviving *Varroa destructor* mite infestation in Sweden for over 10 years without treatment, demonstrate that a balanced host–parasite relationship may evolve over time. Colony-level adaptive traits linked to *Varroa* tolerance were investigated in this population to identify possible characteristics that may be responsible for colony survival in spite of mite infestations. Brood removal rate, adult grooming rate, and the mite distribution between brood and adults were not significantly different in the untreated population compared with treated control colonies. However, colony size and the reproductive success of the mite were significantly reduced in surviving colonies compared with control colonies. Our data suggest that colony-level adaptive traits may limit mite population growth by reducing mite reproduction opportunities and also by suppressing the mite reproductive success.

Apidologie (2011) 42:533–542

Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations

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Ecology and Evolution 2012; 2(6): 1144–1150 doi: 10.1002/ece3.248

Abstract

Honey bee societies (*Apis mellifera*), the ectoparasitic mite *Varroa destructor*, and honey bee viruses that are vectored by the mite, form a complex system of host–parasite interactions. Coevolution by natural selection in this system has been hindered for European honey bee hosts since apicultural practices remove the mite and consequently the selective pressures required for such a process. An increasing mite population means increasing transmission opportunities for viruses that can quickly develop into severe infections, killing a bee colony. Remarkably, a few subpopulations in Europe have survived mite infestation for extended periods of over 10 years without management by beekeepers and offer the possibility to study their natural host–parasite coevolution. Our study shows that two of these “natural” honey bee populations, in Avignon, France and Gotland, Sweden, have in fact evolved resistant traits that reduce the fitness of the mite (measured as the reproductive success), thereby reducing the parasitic load within the colony to evade the development of overt viral infections. Mite reproductive success was reduced by about 30% in both

populations. Detailed examinations of mite reproductive parameters suggest these geographically and genetically distinct populations favor different mechanisms of resistance, even though they have experienced similar selection pressures of mite infestation. Compared to unrelated control colonies in the same location, mites in the Avignon population had high levels of infertility while in Gotland there was a higher proportion of mites that delayed initiation of egg-laying. Possible explanations for the observed rapid coevolution are discussed.

Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies  
Gideon J Mordecai, Laura E Brettell, Stephen J Martin, David Dixon, Ian M Jones and Declan C Schroeder  
The ISME Journal (2015), 1–10

Over the past 50 years, many millions of European honey bee (*Apis mellifera*) colonies have died as the ectoparasitic mite, *Varroa destructor*, has spread around the world. Subsequent studies have indicated that the mite's association with a group of RNA viral pathogens (Deformed Wing Virus, DWV) correlates with colony death. Here, we propose a phenomenon known as superinfection exclusion that provides an explanation of how certain *A. mellifera* populations have survived, despite *Varroa* infestation and high DWV loads. Next-generation sequencing has shown that a non-lethal DWV variant 'type B' has become established in these colonies and that the lethal 'type A' DWV variant fails to persist in the bee population. We propose that this novel stable host-pathogen relationship prevents the accumulation of lethal variants, suggesting that this interaction could be exploited for the development of an effective treatment that minimises colony losses in the future.

The ISME Journal advance online publication, 27 October 2015; doi:10.1038/ismej.2015.186

Increased Tolerance and Resistance to Virus Infections: A Possible Factor in the Survival of *Varroa destructor* Resistant Honey Bees (*Apis mellifera*)

Barbara Locke\*, Eva Forsgren, Joachim R. de Miranda

Citation: Locke B, Forsgren E, de Miranda JR (2014) Increased Tolerance and Resistance to Virus Infections: A Possible Factor in the Survival of *Varroa destructor* Resistant Honey Bees (*Apis mellifera*).

PLoS ONE 9(6): e99998. doi:10.1371/journal.pone.0099998

The honey bee ectoparasitic mite, *Varroa destructor*, has a world-wide distribution and inflicts more damage than all other known apicultural diseases. However, *Varroa*-induced colony mortality is more accurately a result of secondary virus infections vectored by the mite. This means that honey bee resistance to *Varroa* may include resistance or tolerance to virus infections. The aim of this study was to see if this is the case for a unique population of mite-resistant (MR) European honey bees on the island of Gotland, Sweden. This population has survived uncontrolled mite infestation for over a decade, developing specific mite-related resistance traits to do so. Using RT-qPCR techniques, we monitored late season virus infections, *Varroa* mite infestation and honey bee colony population dynamics in the Gotland MR population and compared this to mite-susceptible (MS) colonies in a close by apiary. From summer to autumn the deformed wing virus (DWV) titres increased similarly between the MR and MS populations, while the black queen cell virus (BQCV) and sacbrood virus (SBV) titres decreased substantially in the MR population compared to the MS population by several orders of magnitude. The MR colonies all survived the following winter with high mite infestation, high DWV infection, small colony size and low proportions of autumn brood, while the MS colonies all perished. Possible explanations for these changes in virus titres and their relevance to *Varroa* resistance and colony winter survival are discussed.

Inheritance of reduced *Varroa* mite reproductive success in reciprocal crosses of mite-resistant and mite-susceptible honey bees (*Apis mellifera*)

Barbara LOCKE

Apidologie Original article

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Abstract— A well-documented population of honey bees on Gotland, Sweden is resistant to *Varroa destructor* mites and is able in some way to reduce the mite's reproductive success. The aim of this study was to determine the genetic and maternal contribution to the inheritance of the reduced mite reproductive success trait in this population. Four genotypic groups of colonies were established by crossing the mite-resistant population of Gotland with a mite susceptible population in Uppsala, Sweden, through artificial



insemination of reared queens with drone semen. All the colonies in groups with a genetic origin from the resistant population expressed reduced mite reproductive success regardless if the genetic origin was maternal, paternal or both, and no statistical differences were observed between the reciprocal crosses. These results strongly imply a dominant genetic component to the trait's inheritance, as opposed to maternal effects or epigenetic mechanisms, and that the trait can be easily produced through selective breeding using the mite-resistant Gotland bee stock.

Review: Decomposing health: tolerance and resistance to parasites in animals

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Plant biologists have long recognized that host defence against parasites and pathogens can be divided into two conceptually different components: the ability to limit parasite burden (resistance) and the ability to limit the harm caused by a given burden (tolerance). Together these two components determine how well a host is protected against the effects of parasitism. This distinction is useful because it recognizes that hosts that are best at controlling parasite burdens are not necessarily the healthiest. Moreover, resistance and tolerance can be expected to have different effects on the epidemiology of infectious diseases and host–parasite coevolution. However, studies of defence in animals have to date focused on resistance, whereas the possibility of tolerance and its implications have been largely overlooked. The aim of our review is to (i) describe the statistical framework for analysis of tolerance developed in plant science and how this can be applied to animals, (ii) review evidence of genetic and environmental variation for tolerance in animals, and studies indicating which mechanisms could contribute to this variation, and (iii) outline avenues for future research on this topic.

Phil. Trans. R. Soc. B(2009)364, 37–49, doi:10.1098/rstb.2008.0184, Published online 16 October 2008

Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection

Melissa Oddie, Bjørn Dahle, Peter Neumann PeerJ Preprints |Open Access rec: 12 May 2017, publ: 12 May 2017 <https://peerj.com/articles/3956/>

Background

Managed, feral and wild populations of European honey bee subspecies, *Apis mellifera*, are currently facing severe colony losses globally. There is consensus that the ectoparasitic mite *Varroa destructor*, that switched hosts from the Eastern honey bee *Apis cerana* to the Western honey bee *A. mellifera*, is a key factor driving these losses. For >20 years, breeding efforts have not produced European honey bee colonies that can survive infestations without the need for mite control. However, at least three populations of European honey bees have developed this ability by means of natural selection and have been surviving for >10 years without mite treatments. Reduced mite reproductive success has been suggested as a key factor explaining this natural survival. Here, we report a managed *A. mellifera* population in Norway, that has been naturally surviving consistent *V. destructor* infestations for >17 years.

Methods

Surviving colonies and local susceptible controls were evaluated for mite infestation levels, mite reproductive success and two potential mechanisms explaining colony survival: grooming of adult worker bees and *Varroa Sensitive Hygiene* (VSH): adult workers specifically detecting and removing mite-infested brood.

Results

Mite infestation levels were significantly lower in surviving colonies and mite reproductive success was reduced by 30% when compared to the controls. No significant differences were found between surviving and control colonies for either grooming or VSH.

Discussion

Our data confirm that reduced mite reproductive success seems to be a key factor for natural survival of infested *A. mellifera* colonies. However, neither grooming nor VSH seem to explain colony survival.

Instead, other behaviors of the adult bees seem to be sufficient to hinder mite reproductive success, because brood for this experiment was taken from susceptible donor colonies only. To mitigate the global impact of *V. destructor*, we suggest learning more from nature, i.e., identifying the obviously efficient mechanisms favored by natural selection.

Locke, B. (2016) Natural Varroa mite-surviving *Apis mellifera* honeybee populations. *Apidologie* 47, 467-482. <https://hal.archives-ouvertes.fr/hal-01532364/document>

The Varroa destructor mite is the largest threat to apiculture worldwide and has been responsible for devastating losses of wild honeybee populations in Europe and North America. However, Varroa mite-resistant populations of *A. mellifera* honeybees have been reported and documented around the world with a variety of explanations for their long-term survival with uncontrolled mite infestation. This review synthesizes the work on naturally occurring survival to Varroa mites and discusses what these honeybee populations can signify for apiculture.

Oldest Varroa tolerant honey bee population provides insight into the origins of the global decline of honey bees.

Brettell LE, Martin SJ

Sci Rep. 2017 Apr 10;7:45953. doi: 10.1038/srep45953.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5385554/>

The ecto-parasitic mite *Varroa destructor* has transformed the previously inconsequential Deformed Wing Virus (DWV) into the most important honey bee viral pathogen responsible for the death of millions of colonies worldwide. Naturally, DWV persists as a low level covert infection transmitted between nest-mates. It has long been speculated that *Varroa* via immunosuppression of the bees, activate a covert infection into an overt one. Here we show that despite *Varroa* feeding on a population of 20-40 colonies for over 30 years on the remote island of Fernando de Noronha, Brazil no such activation has occurred and DWV loads have remained at borderline levels of detection. This supports the alternative theory that for a new vector borne viral transmission cycle to start, an outbreak of an overt infection must first occur within the host. Therefore, we predict that this honey bee population is a ticking time-bomb, protected by its isolated position and small population size. This unique association between mite and bee persists due to the evolution of low *Varroa* reproduction rates. So the population is not adapted to tolerate *Varroa* and DWV, rather the viral quasispecies has simply not yet evolved the necessary mutations to produce a virulent variant.

John Kefuss, Jacques Vanpoucke, Maria Bolt & Cyril Kefuss (2016): Selection for resistance to *Varroa destructor* under commercial beekeeping conditions, *Journal of Apicultural Research*,

<http://dx.doi.org/10.1080/00218839.2016.1160709>

A survival field test was initiated in 1999 to observe the effects of no treatment against *Varroa destructor* on European honey bee colony survival. After losses of over two-thirds of the 268 original colonies, new colonies were made from the survivors. In 2002, genetic material from these survivors was bred into an independent group of 60 colonies. In 2013, 519 non-treated colonies from both groups were being used for commercial beekeeping, and mite populations were very low. This indicates that under commercial beekeeping conditions, simple methods can be used to select for reduced mite populations.

Brettell, LE and Martin, SJ 2017, 'Oldest *Varroa* tolerant honey bee population provides insight into the origins of the global decline of honey bees', *Scientific Reports*. (In Press) Abstract only:

<http://usir.salford.ac.uk/41567/> When this eventually comes out in 'Nature Scientific Reports', it will be interesting to see how they dated it as the 'oldest' and clarify some other features of the seemingly speculative abstract.

Temporal changes in the viromes of Swedish *Varroa*-resistant and *Varroa*-susceptible honeybee populations a.. Srinivas Thaduri, b.. Barbara Locke, c.. Fredrik Granberg, d.. Joachim R. de Miranda Published: December 6, 2018

PLoS ONE 13(12): e0206938.

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0206938>

The parasitic mite, *Varroa destructor*, in combination with the viruses it vectors, is the main cause for global colony losses of the European honeybee, *Apis mellifera*. However, an isolated honeybee population established in 1999 on the Island of Gotland, Sweden has naturally acquired resistance to the mite, and has survived without mite control treatment for more than 18 years. A recent study has shown that this mite resistant (MR) population also appears to be resistant to Black queen cell virus (BQCV) and Sacbrood virus (SBV) and tolerant to Deformed wing virus (DWV), relative to nearby mite susceptible (MS) honeybee populations. In this study, RNA sequencing was employed to corroborate these previous findings and identify other viral factors that may play a role in the enhanced survival of this mite resistant honeybee population. Two additional honeybee-infecting viruses, Apis rhabdovirus-1 (ARV-1) and Lake Sinai virus (LSV), were identified and near-complete genomes of these two viruses were obtained. Phylogenetic analyses of the assembled virus sequences revealed consistent separation between the MR and MS honeybee populations, although it is unclear whether this is due to pre-existing differences between the viruses in the two populations when they were established, and isolated, or due to virus genetic adaptation towards reduced virulence in the MR population, to promote colony survival. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analyses show higher ARV and LSV titres in MS colonies compared to MR colonies, gradually increasing from summer to autumn 2009, and reaching maximum titres in the following spring 2010. While the DWV and BQCV titres in MR colonies increased between autumn 2009 and spring 2010, the SBV practically disappeared entirely by spring 2010. Possible explanations for the apparent virus tolerance or resistance in the Gotland mite-resistant honeybee population are discussed.

Reduced Postcapping Period in Honey Bees Surviving *Varroa destructor* by Means of Natural Selection  
M Oddie, B Dahle, P Neumann - *Insects*, 2018

*Insects* 2018, 9, 149; doi:10.3390/insects9040149

<https://www.mdpi.com/2075-4450/9/4/149/pdf>

The ectoparasitic mite *Varroa destructor* is a key factor for colony losses in European honey bee subspecies (*Apis mellifera*), but it is also known that some host populations have adapted to the mite by means of natural selection. The role of a shorter host brood postcapping period in reducing mite reproductive success has been investigated in other surviving subspecies, however its role in the adaptation of European honey bee populations has not been addressed. Here, we use a common garden approach to compare the length of the worker brood postcapping period in a Norwegian surviving honey bee population with the postcapping period of a local susceptible population. The data show a significantly shorter postcapping period in the surviving population for ~10% of the brood. Since even small differences in postcapping period can significantly reduce mite reproductive success, this mechanism may well contribute to natural colony survival. It appears most likely that several mechanisms acting together produce the full mite-surviving colony phenotype.

<https://doi.org/10.1080/0005772X.2018.1431000> Adaptation in Honey Bee (*Apis mellifera*) Colonies Exhibiting Tolerance to *Varroa destructor* in Ireland

John McMullan (2018)

*Bee World*, 95:2, 39-43, DOI: 10.1080/0005772X.2018.1431000

NO ABSTRACT AVAILABLE

Rapid parallel evolution overcomes global honey bee parasite  
Melissa Oddie, Ralph B uchler, Bj orn Dahle, Marin Kovacic, Yves Le Conte, Barbara Locke, Joachim R. de Miranda, Fanny Mondet & Peter Neumann  
*Scientific Reports* volume 8, Article number: 7704 (2018) doi:10.1038/s41598-018-26001-7

<https://www.nature.com/articles/s41598-018-26001-7>

In eusocial insect colonies nestmates cooperate to combat parasites, a trait called social immunity. However, social immunity failed for Western honey bees (*Apis mellifera*) when the ectoparasitic mite *Varroa destructor* switched hosts from Eastern honey bees (*Apis cerana*). This mite has since become the most severe threat to *A. mellifera* world-wide. Despite this, some isolated *A. mellifera* populations are known to survive infestations by means of natural selection, largely by suppressing mite reproduction, but the underlying mechanisms of this are poorly understood. Here, we show that a cost-effective social immunity mechanism has evolved rapidly and independently in four naturally *V. destructor*-surviving *A. mellifera* populations. Worker bees of all four ‘surviving’ populations uncapped/recapped worker brood cells more frequently and targeted mite-infested cells more effectively than workers in local susceptible colonies.

Direct experiments confirmed the ability of uncapping/recapping to reduce mite reproductive success without sacrificing nestmates. Our results provide striking evidence that honey bees can overcome exotic parasites with simple qualitative and quantitative adaptive shifts in behaviour. Due to rapid, parallel evolution in four host populations this appears to be a key mechanism explaining survival of mite infested colonies.

<https://www.nature.com/articles/s41598-019-45008-2>

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

K. Wagoner, M. Spivak, A. Hefetz, T. Reams & O. Rueppell

Scientific Reports volume 9, Article number: 8753 (2019)

The health of the honey bee *Apis mellifera* is challenged by the ectoparasitic mite *Varroa destructor*, and the numerous harmful pathogens it vectors. Existing pesticide-based *Varroa* controls are not sustainable. In contrast, one promising approach for improved honey bee health is the breeding of hygienic bees, capable of detecting and removing brood that is parasitized or diseased. In three experiments we find evidence to support the hypothesis that stock-specific chemical brood signals are induced by *Varroa* and Deformed Wing Virus, and elicit hygienic response in the honey bee. By collecting, analyzing, and running bioassays involving mite-infested and control brood extracts from three honey bee breeding stocks we: 1) found evidence that a transferrable chemical signal for hygienic behavior is present in *Varroa*-infested brood extracts, 2) identified ten stock-specific hydrocarbons as candidates of hygienic signaling, and 3) found that two of these hydrocarbons linked to *Varroa* and DWV were also elevated in brood targeted for hygienic behavior. These findings expand our understanding of honey bee chemical communication, and facilitate the development of improved hygienic selection tools to breed honey bees with greater resistance to *Varroa* and associated pathogens.

Disentangling host-parasite-pathogen interactions in a varroa-resistant honeybee population reveals virus tolerance as an independent, naturally adapted survival mechanism

Srinivas Thaduri, Jörg G. Stephan, Joachim R. de Miranda & Barbara Locke

Scientific Reports volume 9, Article number: 6221 (2019)

<https://www.nature.com/articles/s41598-019-42741-6>

The ectoparasitic mite, *Varroa destructor*, is unarguably the leading cause of honeybee (*Apis mellifera*) mortality worldwide through its role as a vector for lethal viruses, in particular, strains of the Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV) complexes. This multi-level system of host-parasite-pathogen interactions makes it difficult to investigate effects of either the mite or the virus on natural host survival. The aim of this study was to remove confounding effects of varroa to examine the role of virus susceptibility in the enhanced survival of a naturally adapted Swedish mite-resistant (MR) honeybee population, relative to mite-susceptible (MS) honeybees. Caged adult bees and laboratory reared larvae, from varroa-free colonies, were inoculated with DWV and ABPV in a series of feeding infection experiments, while control groups received virus-free food. Virus infections were monitored using RT-qPCR assays in individuals sampled over a time course. In both adults and larvae the DWV and ABPV infection dynamics were nearly identical between MR and MS groups, but MS adults suffered significantly higher mortality than MR adults. Results suggest virus tolerance, rather than reduced susceptibility or virus resistance, is an important component of the natural survival of this honeybee population.

<http://www.tandfonline.com/doi/pdf/10.1080/00218839.2017.1351860?needAccess=true>

Delphine Panziera, Frank van Langevelde & Tjeerd Blacquière (2017) *Varroa* sensitive hygiene contributes to naturally selected varroa resistance in honey bees,

Journal of Apicultural Research, 56:5, 635-642, DOI: 10.1080/00218839.2017.1351860

The parasitic mite *Varroa destructor* is a serious threat for western honey bee colonies and beekeepers are compelled to control it to keep their colonies healthy. Yet, by controlling varroa no resistance to the parasite can evolve. As a trial, honey bee colonies have been left untreated in isolated locations to allow development of resistance or tolerance to the mite. These colonies developed an ability to live without control measures against varroa, although the traits responsible for this resistance or tolerance are still unclear. Two of these resistant populations have been studied to test the involvement of specific varroa mite targeted hygienic behaviour varroa sensitive hygiene (VSH) in the acquired resistance. Individual mites

were manually introduced into just capped brood cells, after which the brood combs were placed in colonies of the two resistant populations and in control colonies in which varroa had always been controlled. We followed the development of the mites, including possible removals. We found that VSH had increased strongly in one of the selections, up to 40% of the infested cells with mites and pupae were removed, but it had decreased in the other selection, compared to the control colonies. Further we could not conclude from our data that VSH only or preferentially targets reproducing mites, leaving non-reproducing mites undisturbed. The different VSH responses between the two selected resistant honey bee populations lead to conclude that more than one mechanism of resistance may evolve in response to the selection pressure by varroa mites.

Biological Invasions, pp 1–10, First Online: 16 May 2019

Darwinian black box selection for resistance to settled invasive *Varroa destructor* parasites in honey bees  
Tjeerd Blacquière, Willem Boot, Johan Calis, Arrigo Moro, Peter Neumann, Delphine Panziera,  
<https://link.springer.com/article/10.1007/s10530-019-02001-0>

Established invasive species can pose a continuous threat to biodiversity and food security, thereby calling for sustainable mitigation. There is a consensus that the ubiquitous ecto-parasitic mite *Varroa destructor*, an invasive species from Asia, is the main biological threat to global apiculture with *Apis mellifera*. *V. destructor* has almost completely wiped out wild European honey bee (*Apis mellifera*) populations. The only remedy for apiculture, to date, is frequent control measures against the mite throughout the season, which prevents possible adaptations. While targeted breeding efforts have, so far, not achieved the selection of tolerant or resistant bees, natural selection approaches have succeeded at least seven times. Here, we propose to take advantage of natural selection for honey bee resistance by stopping mite treatment in managed colonies. The main principles are within population mating of the colonies' own virgin queens and drones and selection based on survival and proliferous development of colonies. Being used for 10 years, it has shown to result in *grosso modo* 'normal' colonies with a high level of resistance to *V. destructor*. Here, we call for local groups of beekeepers and scientists to join a novel natural selection program that has started so far on three locations. This will eventually lead to several locally adapted *V. destructor* resistant honey bee populations around the world, and help global apiculture becoming more sustainable.

## **Pesticide residues including acaricides (miticides) in brood comb, beeswax, foundation etc.**

Kakumanu M, Reeves AM, Anderson T, Rodrigues RR and Williams MA(2016)

Honey bee gut microbiome is altered by in-hive pesticide exposures.

Front. Microbiol.7:1255. doi:10.3389/fmicb.2016.01255

Honey bees (*Apis mellifera*) are the primary pollinators of major horticultural crops. Over the last few decades, a substantial decline in honey bees and their colonies have been reported. While a plethora of factors could contribute to the putative decline, pathogens, and pesticides are common concerns that draw attention. In addition to potential direct effects on honey bees, indirect pesticide effects could include alteration of essential gut microbial communities and symbionts that are important to honey bee health (e.g., immune system). The primary objective of this study was to determine the microbiome associated with honey bees exposed to commonly used in-hive pesticides: coumaphos, tau-fluvalinate, and chlorothalonil. Treatments were replicated at three independent locations near Blacksburg Virginia, and included a no-pesticide amended control at each location. The microbiome was characterized through pyrosequencing of V2–V3 regions of the bacterial 16S rRNA gene and fungal ITS region. Pesticide exposure significantly affected the structure of bacterial but not fungal communities. The bee bacteriome, similar to other studies, was dominated by sequences derived from Bacilli, Actinobacteria,  $\alpha$ -,  $\beta$ -,  $\gamma$ -proteobacteria. The fungal community sequences were dominated by Ascomycetes and Basidiomycetes. The Multi-response permutation procedures (MRPP) and subsequent Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) analysis indicated that chlorothalonil caused significant change to the structure and functional potential of the honey bee gut bacterial community relative to control. Putative genes for oxidative phosphorylation, for example, increased while sugar metabolism and peptidase potential declined in the microbiome of chlorothalonil exposed bees. The results of this field-based study

suggest the potential for pesticide induced changes to the honey bee gut microbiome that warrant further investigation.

**COMMENT: This potential background contamination of the hive can be minimised by using natural comb.**

Residues in wax and honey after Apilife VAR; treatment

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(Received 3 April 1998; accepted 7 July 1998)

Abstract -

Apilife VAR; with thymol as its main active ingredient, is registered for use against *Varroa jacobsoni* Oudemans in Switzerland. After Apilife VAR; treatment in autumn of 1992, the residues in honey and comb were examined the following spring. Only thymol residues were found in honey, whereas in comb the residues consisted of 99 % thymol and 1 % menthol. The thymol residues in honey did not increase with an increasing number of treatments and varied between 0.02 to 0.48 mg·kg<sup>-1</sup> with an average of 0.15 mg·kg<sup>-1</sup> (n = 29). The taste threshold of thymol in acacia and rape honey was between 1.1 and 1.6 mg·kg<sup>-1</sup>. The brood comb in two apiaries, where Apilife VAR; was used for, on average, 4 consecutive years, had a mean content of 574 mg·kg<sup>-1</sup> and this did not increase with an increasing number of treatments. The thymol residues in honey comb were on average 21.6 mg·kg<sup>-1</sup>. Thymol did not evaporate during comb melting, but decreased rapidly when comb and foundation were exposed to the air during storage. © Inra/DIB/AGIB/Elsevier, Paris

Determination of residues in honey after treatments with formic and oxalic acid under field conditions

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Verena KILCHENMANN, Peter FLURI

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(Received 30 August 2001; revised 4 February 2002; accepted 1 March 2002)

Abstract—

Formic acid and oxalic acid field trials for control of *Varroa destructor* were carried out in autumn according to the Swiss prescriptions during three successive years in different apiaries in Switzerland. The following parameters were determined in honey that was harvested the year after treatment: formic acid, oxalic acid and free acidity. The following range of values were found in honeys of untreated colonies: formic acid, from 17 to 284 mg/kg, n = 34; oxalic acid, from 11 to 119 mg/kg, n = 33. There was a small, but unproblematic increase in formic acid levels in comparison to the levels in the controls; average: 46 mg/kg, maximum: 139 mg/kg. No increase in formic acid was found with increasing number of treatment years. If emergency formic acid treatments were carried out in spring, the residue levels were much higher: average increase of 193 mg/kg, maximum 417 mg/kg. The oxalic acid content remained unchanged, even after two successive treatments during the same autumn. No rise of free acidity was encountered after a combined treatment with formic and oxalic acid during the three trial years.

Apidologie 33 (2002) 399–409

© INRA/DIB-AGIB/EDP Sciences, 2002

DOI: 10.1051/apido:2002029

Journal of Apicultural Research 43(1): 14–16(2004) © IBRA 2004

Residues of para-dichlorobenzene in honey and beeswax

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Received 29 July 2003, accepted subject to revision 17 November 2003, accepted for publication

21 November 2003

SUMMARY

Para-dichlorobenzene (PDCB) is an insecticide used in beekeeping for wax moth control. Analysis of PDCB residues were carried out on Swiss retail market honey samples by the cantonal food control authorities in 1997, 1998, 2000, 2001 and 2002. 173 Swiss honeys and 287 imported samples were analysed. On average, 30% of the Swiss honeys contained PDCB, 13% of them being above the Swiss tolerance value of 10 µg/kg. On the other hand, only 7% of the imported honeys were contaminated. The minimum values were 2 µg/kg, the maximum ones 112 µg/kg. Long-term monitoring of Swiss beeswax, carried out from 1993 to 2000, showed that most of the comb foundation beeswax produced in Switzerland is contaminated by PDCB with values ranging from one to 60 mg/kg. The results show that the reason for this contamination is the use of PDCB for the control of wax moth. These residues can be avoided as wax moth can be controlled successfully with alternative methods, carried out according to good apicultural practice.

Keywords: para-dichlorobenzene, residues, analysis, *Galleria mellonella*, wax moth control, honey, beeswax,

*Apidologie* 37 (2006) 1–18

© INRA/DIB-AGIB/ EDP Sciences, 2005

DOI: 10.1051/apido:2005043

Review article: Contaminants of bee products

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Received 22 July 2004 – revised 22 December 2004 – accepted 26 January 2005

Abstract –

Bee products can be contaminated from different sources. The contamination can arise from beekeeping practices or from the environment. Environmental contaminants are covered in the first part of the review. They are: the heavy metals lead, cadmium and mercury, radioactive isotopes, organic pollutants, pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria and genetically modified organisms. The second part of the review discusses contaminants from beekeeping. The main ones are acaricides: lipophilic synthetic compounds and non-toxic substances such as organic acids and components of essential oils; and antibiotics used for the control of bee brood diseases, mainly tetracyclines, streptomycine, sulfonamides and chloramphenicol. Other substances used in beekeeping play a minor role: para-dichlorobenzene, used for the control of wax moth and chemical repellents. The degree of contamination of honey, pollen, beeswax, propolis and royal jelly by the different contaminants is reviewed.

*Pest Management Science* *Pest Manag Sci* 63:1100–1106 (2007)

Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France

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Abstract:

In 2002 a field survey was initiated in French apiaries in order to monitor the health of honey bee colonies (*Apis mellifera* L.). Studied apiaries were evenly distributed across five sites located in continental France. Beeswax samples were collected once a year over 2 years from a total of 125 honey bee colonies. Multiresidue analyses were performed on these samples in order to identify residues of 16 insecticides and acaricides and two fungicides. Residues of 14 of the searched-for compounds were found in samples. Tau-fluvalinate, coumaphos and endosulfan residues were the most frequently occurring residues (61.9, 52.2 and 23.4% of samples respectively). Coumaphos was found in the highest average quantities (792.6 µg kg<sup>-1</sup>). Residues of cypermethrin, lindane and deltamethrin were found in 21.9, 4.3 and 2.4% of samples respectively. Statistical tests showed no difference between years of sampling, with the exception of the frequency of pyrethroid residues. Beeswax contamination was the result of both in-hive acaricide treatments and, to a much lesser extent, environmental pollution.

<sup>□</sup> 2007 Society of Chemical Industry

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© INRA/DIB-AGIB/EDP Sciences, 2008 [www.apidologie.org](http://www.apidologie.org)

DOI: 10.1051/apido:2008012

Acaricide residues in beeswax after conversion to organic beekeeping methods\*

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Received 22 June 2007 – Revised 21 December 2007 – Accepted 17 January 2008

Abstract–

Beekeepers interested in converting their honey farms to organic management must replace old combs with organic foundations. The experiment described in this paper compares two methods of replacement of old combs, “fast” (5 combs per year) and “slow” (2 combs per year), by measuring the levels of acaricide residues in the newly built combs. Tested acaricides were coumaphos (Perizin and Asuntol), fluvalinate (Apistan), and chlorfenvinphos (Supona). Significant differences between the two replacement groups were observed only for the Apistan group in the third year, confirming high lipophilicity of fluvalinate. The residue levels in the new combs three years after beginning the conversion were significantly lower than initial levels for all products. Direct contamination of the combs was highest in Asuntol-treated hives and lowest in Perizin-treated hives. Residues in honey exceeding EU Maximum Residue Limit were found only in the case of Asuntol.

Short Communication

“Entombed Pollen”: A new condition in honey bee colonies associated with increased risk of colony mortality

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e Florida Department of Agriculture and Consumer Services, 1911 South West 34th Street, P.O. Box 147100, Gainesville, FL 32614-7100, United States

Abstract

Here we describe a new phenomenon, entombed pollen, which is highly associated with increased colony mortality. Entombed pollen is sunken, capped cells amidst “normal”, uncapped cells of stored pollen, and some of the pollen contained within these cells is brick red in color. There appears to be a lack of microbial agents in the pollen, and larvae and adult bees do not have an increased rate of mortality when they are fed diets supplemented with entombed pollen in vitro, suggesting that the pollen itself is not directly responsible for increased colony mortality. However, the increased incidence of entombed pollen in reused wax comb suggests that there is a transmittable factor common to the phenomenon and colony mortality. In addition, there were elevated pesticide levels, notably of the fungicide chlorothalonil, in entombed pollen. Additional studies are needed to determine if there is a causal relationship between entombed pollen, chemical residues, and colony mortality.

Journal of Invertebrate Pathology 101 (2009) 147–149

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Review article: Pesticides and honey bee toxicity – USA\*

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Abstract– Until 1985 discussions of pesticides and honey bee toxicity in the USA were focused on pesticides applied to crops and the unintentional exposure of foraging bees to them. The recent introduction of arthropod pests of honey bees, *Acarapis woodi* (1984), *Varroa destructor* (1987), and *Aethina tumida* (1997), to the USA have resulted in the intentional introduction of pesticides into beehives to suppress these pests. Both the unintentional and the intentional exposure of honey bees to pesticides have resulted in residues in



hive products, especially beeswax. This review examines pesticides applied to crops, pesticides used in apiculture and pesticide residues in hive products. We discuss the role that pesticides and their residues in hive products may play in colony collapse disorder and other colony problems. Although no single pesticide has been shown to cause colony collapse disorder, the additive and synergistic effects of multiple pesticide exposures may contribute to declining honey bee health.

#### Acaricide, Fungicide and Drug Interactions in Honey Bees (*Apis mellifera*)

Johnson RM, Dahlgren L, Siegfried BD, Ellis MD (2013) Acaricide, Fungicide and Drug Interactions in Honey Bees (*Apis mellifera*). PLoS ONE 8(1): e54092. doi:10.1371/journal.pone.0054092

**Background:** Chemical analysis shows that honey bees (*Apis mellifera*) and hive products contain many pesticides derived from various sources. The most abundant pesticides are acaricides applied by beekeepers to control *Varroa destructor*. Beekeepers also apply antimicrobial drugs to control bacterial and microsporidial diseases. Fungicides may enter the hive when applied to nearby flowering crops. Acaricides, antimicrobial drugs and fungicides are not highly toxic to bees alone, but in combination there is potential for heightened toxicity due to interactive effects.

**Methodology/Principal Findings:** Laboratory bioassays based on mortality rates in adult worker bees demonstrated interactive effects among acaricides, as well as between acaricides and antimicrobial drugs and between acaricides and fungicides. Toxicity of the acaricide tau-fluvalinate increased in combination with other acaricides and most other compounds tested (15 of 17) while amitraz toxicity was mostly unchanged (1 of 15). The sterol biosynthesis inhibiting (SBI) fungicide prochloraz elevated the toxicity of the acaricides tau-fluvalinate, coumaphos and fenpyroximate, likely through inhibition of detoxicative cytochrome P450 monooxygenase activity. Four other SBI fungicides increased the toxicity of tau-fluvalinate in a dose-dependent manner, although possible evidence of P450 induction was observed at the lowest fungicide doses. Non-transitive interactions between some acaricides were observed. Sublethal amitraz pre-treatment increased the toxicity of the three P450-detoxified acaricides, but amitraz toxicity was not changed by sublethal treatment with the same three acaricides. A two-fold change in the toxicity of tau-fluvalinate was observed between years, suggesting a possible change in the genetic composition of the bees tested.

**Conclusions/Significance:** Interactions with acaricides in honey bees are similar to drug interactions in other animals in that P450-mediated detoxication appears to play an important role. Evidence of non-transitivity, year-to-year variation and induction of detoxication enzymes indicates that pesticide interactions in bees may be as complex as drug interactions in mammals.

#### High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health

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Pennsylvania, United States of America, <sup>4</sup> Bee Research Laboratory, United States Department of Agriculture - Agricultural Research Service, Beltsville, Maryland, United

States of America

#### Abstract

**Background:** Recent declines in honey bees for crop pollination threaten fruit, nut, vegetable and seed production in the United States. A broad survey of pesticide residues was conducted on samples from migratory and other beekeepers across 23 states, one Canadian province and several agricultural cropping systems during the 2007–08 growing seasons.

**Methodology/Principal Findings:** We have used LC/MS-MS and GC/MS to analyze bees and hive matrices for pesticide residues utilizing a modified QuEChERS method. We have found 121 different pesticides and metabolites within 887 wax, pollen, bee and associated hive samples. Almost 60% of the 259 wax and 350 pollen samples contained at least one systemic pesticide, and over 47% had both in-hive acaricides fluvalinate and coumaphos, and chlorothalonil, a widely-used fungicide. In bee pollen were found chlorothalonil at levels up to 99 ppm and the insecticides aldicarb, carbaryl, chlorpyrifos and imidacloprid, fungicides boscalid, captan and myclobutanil, and herbicide pendimethalin at 1 ppm levels. Almost all comb

and foundation wax samples (98%) were contaminated with up to 204 and 94 ppm, respectively, of fluvalinate and coumaphos, and lower amounts of amitraz degradates and chlorothalonil, with an average of 6 pesticide detections per sample and a high of 39. There were fewer pesticides found in adults and brood except for those linked with bee kills by permethrin (20 ppm) and fipronil (3.1 ppm).

Conclusions/Significance: The 98 pesticides and metabolites detected in mixtures up to 214 ppm in bee pollen alone represents a remarkably high level for toxicants in the brood and adult food of this primary pollinator. This represents over half of the maximum individual pesticide incidences ever reported for apiaries. While exposure to many of these neurotoxicants elicits acute and sublethal reductions in honey bee fitness, the effects of these materials in combinations and their direct association with CCD or declining bee health remains to be determined.

Citation: Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. (2010) High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. PLoS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

### Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity

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Abstract

Background: Numerous surveys reveal high levels of pesticide residue contamination in honey bee comb. We conducted studies to examine possible direct and indirect effects of pesticide exposure from contaminated brood comb on developing worker bees and adult worker lifespan.

Methodology/Principal Findings: Worker bees were reared in brood comb containing high levels of known pesticide residues (treatment) or in relatively uncontaminated brood comb (control). Delayed development was observed in bees reared in treatment combs containing high levels of pesticides particularly in the early stages (day 4 and 8) of worker bee development. Adult longevity was reduced by 4 days in bees exposed to pesticide residues in contaminated brood comb during development. Pesticide residue migration from comb containing high pesticide residues caused contamination of control comb after multiple brood cycles and provided insight on how quickly residues move through wax. Higher brood mortality and delayed adult emergence occurred after multiple brood cycles in contaminated control combs. In contrast, survivability increased in bees reared in treatment comb after multiple brood cycles when pesticide residues had been reduced in treatment combs due to residue migration into uncontaminated control combs, supporting comb replacement efforts. Chemical analysis after the experiment confirmed the migration of pesticide residues from treatment combs into previously uncontaminated control comb.

Conclusions/Significance: This study is the first to demonstrate sub-lethal effects on worker honey bees from pesticide residue exposure from contaminated brood comb. Sub-lethal effects, including delayed larval development and adult emergence or shortened adult longevity, can have indirect effects on the colony such as premature shifts in hive roles and foraging activity. In addition, longer development time for bees may provide a reproductive advantage for parasitic *Varroa destructor* mites. The impact of delayed development in bees on *Varroa* mite fecundity should be examined further.

Citation: Wu JY, Anelli CM, Sheppard WS (2011) Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity. PLoS ONE 6(2): e14720. doi:10.1371/journal.pone.0014720

### Killing Them with Kindness? In-Hive Medications May Inhibit Xenobiotic Efflux Transporters and Endanger Honey Bees

David J. Hawthorne\*, Galen P. Dively

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Abstract

Background: Honey bees (*Apis mellifera*) have recently experienced higher than normal overwintering colony losses. Many factors have been evoked to explain the losses, among which are the presence of residues of pesticides and veterinary products in hives. Multiple residues are present at the same time, though most often in low concentrations so that no single product has yet been associated with losses. Involvement of a combination of residues to losses may however not be excluded. To understand the impact

of an exposure to combined residues on honey bees, we propose a mechanism-based strategy, focusing here on Multi-Drug Resistance (MDR) transporters as mediators of those interactions.

**Methodology/Principal Findings:** Using whole-animal bioassays, we demonstrate through inhibition by verapamil that the widely used organophosphate and pyrethroid acaricides coumaphos and t-fluvalinate, and three neonicotinoid insecticides: imidacloprid, acetamiprid and thiacloprid are substrates of one or more MDR transporters. Among the candidate inhibitors of honey bee MDR transporters is the in-hive antibiotic oxytetracycline. Bees prefed oxytetracycline were significantly sensitized to the acaricides coumaphos and t-fluvalinate, suggesting that the antibiotic may interfere with the normal excretion or metabolism of these pesticides.

**Conclusions/Significance:** Many bee hives receive regular treatments of oxytetracycline and acaricides for prevention and treatment of disease and parasites. Our results suggest that seasonal co-application of these medicines to bee hives could increase the adverse effects of these and perhaps other pesticides. Our results also demonstrate the utility of a mechanism-based strategy. By identifying pesticides and apicultural medicines that are substrates and inhibitors of xenobiotic transporters we prioritize the testing of those chemical combinations most likely to result in adverse interactions.

**Citation:** Hawthorne DJ, Dively GP (2011) Killing Them with Kindness? In-Hive Medications May Inhibit Xenobiotic Efflux Transporters and Endanger Honey Bees. PLoS ONE 6(11): e26796. doi:10.1371/journal.pone.0026796

## **Toxicity of acaricides used against Varroa**

Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud.  
Mariano Higes Aránzazu Meana Miguel Suárez Jesús Llorente

**Abstract**

Two oxalic acid treatments were given to five colonies in autumn and five colonies in spring. In each treatment, colonies were treated every 7 days for 4 weeks with a 3 % sprayed oxalic acid. Another five colonies in each season served as controls and were sprayed only with water. Efficacy of oxalic acid in autumn was 94 % and in spring was 73 %. A long-term study of the colonies for 3-4 months after the last application of oxalic acid showed a statistically significant negative effect of the acid on brood development. In addition, three queens died in the treated colonies.

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Short term negative effect of oxalic acid in *Apis mellifera iberiensis*

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**Abstract**

The toxic effect of oxalic acid (OA) on *Apis mellifera iberiensis* was studied using field and laboratory assays. Bee deaths were higher in OA treated hives than in control hives. Pathological repercussions of topical application of 10% OA were observed in different internal honeybee organs. After 24 h, there were severe alterations in the ventricular epithelial layer while by 48 h there was clearly seen degeneration of the rectal epithelium. Irreversible lesions appeared at 48 h in different bee organs with increased cellular damage after 72 h. Indications are that the effect of the OA continues after initial contact and causes permanent lesions in digestive and excretory organs. Tissue distribution of the acid in different bee organs, after topical administration, suggests that some of the acid is ingested, in some way, by the bee.

*Spanish Journal of Agricultural Research* 2007 5(4), 474-480

Cell death in honeybee (*Apis mellifera*) larvae treated with oxalic or formic acid

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Abstract – The effects of oxalic (OA) and formic acids (FA) on honeybee larvae in colonies were assessed and evaluated. Cell death was detected by the TUNEL technique for DNA labelling. In 3- and 5-day-old larvae exposed to OA, cell death was found in 25% of midgut epithelial cells 5 h after the treatment, using an “In situ cell death detection kit, AP” (Roche). The level of cell death increased to 70% by the 21st hour and the morphology of the epithelium remained unchanged. Fifty hours after the application, cell death was established in 18% of the epithelial cells of the 3-day-old larvae and had increased to 82% in the 5-day-old larvae. A “DeadEnd” apoptosis detection kit (Promega) showed sporadic cell death mainly in the larval fat body 5 h after treatment. Twenty-one hours after the OA application cell death was found in 4% of the larval midgut epithelial cells. Evaporated formic acid induced extensive apoptotic cell death in the peripheral, cuticular and subcuticular tissues that preceded the cell death of the entire larval body. *Apis mellifera* / cell death / oxalic acid / formic acid / immunochemical method  
Apidologie 35 (2004) 453–460

The influence of formic acid on the body surface proteolytic system at different developmental stages in *Apis mellifera* L. workers

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#### Summary

To verify the hypothesis that formic acid (FA) has a suppressive effect on the proteolytic system of *Apis mellifera* cuticle depending on the developmental stage of the insects, 1 to 4-day-old larvae, 8-day-old larvae, pupae, 1-day-old workers, and foragers were sampled from FA treated and untreated colonies for seven weeks. Hydrophilic (H<sup>+</sup>) and hydrophobic protein (H<sup>-</sup>) solutions were washed out from the sampled individual body surfaces. Subsequently, protein concentration, protease activities and protease inhibitor activities were determined. Antifungal and antibacterial activities were also determined. Two-week exposure to FA increased H<sup>+</sup> and H<sup>-</sup> protein concentration but when it was prolonged to three to seven weeks, the concentration fell below the level of untreated colonies. FA treatment decreased H<sup>+</sup> protease activities in workers and larvae, but increased them in pupae. H<sup>-</sup> protease activities oscillated (destabilisation) up and down relative to the control level which was steady. Asparagine and serine proteases were present on apian cuticles independently of the developmental stage, while FA application additionally activated thiolic proteases. FA treatment considerably decreased both H<sup>+</sup> and H<sup>-</sup> natural protease inhibitor activities in larvae and pupae but mostly increased them in workers. Antifungal and antibacterial activities of the body surface washings (in vivo microbiological tests) were suppressed in workers and larvae treated with FA. FA treatment may suppress proteolytic resistance of the bee cuticle but the responses of larvae and workers are different.

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DOI 10.3896/IBRA.1.51.3.06

Schneider, S., Eisenhardt, D., Rademacher, E. (2011) Sublethal effects of oxalic acid on *Apis mellifera* L. (Hymenoptera: Apidae): changes in behaviour and longevity, *Apidologie* DOI: 10.1007/s13592-011-0102-0  
Possibly this PDF: [www.nand.be/ambrosius/nieuws/oxaalzuurtest.pdf](http://www.nand.be/ambrosius/nieuws/oxaalzuurtest.pdf)

#### Abstract

Oxalic acid dehydrate (OA) in the application form of trickling (3.5% solution) has shown a good bee tolerance. However, negative long-term effects of the treatment on honeybees are expected. The sublethal effects of OA on division of labour, activity, olfactory learning and the longevity of *Apis mellifera* were studied. Newly emerged workers were treated with 3.5% OA solution by topical application (dosage 175 µg/bee) and introduced into a colony. Behavioural observations were carried out and the longevity of every worker was recorded. To investigate the learning behaviour, foragers were trained in a classical olfactory conditioning paradigm, the olfactory conditioning of the proboscis extension response. These experiments revealed sublethal effects of OA on *Apis mellifera*. The treatment caused a significant decrease in worker activity, nursing behaviour and longevity. Treated bees also showed significantly more self-grooming and a higher response in the olfactory conditioning than bees of the control group.

European Journal of Scientific Research

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Evaluation of Secondary Effects of some Acaricides on *Apis Mellifera Intermissa* (Hymenoptera, Apidae):  
Acetylcholinesterase and Glutathione S-Transferase Activities

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Abstract

The parasitic mite *Varroa destructor* (Acari, Varroidae) is a serious world-wide pest of the honeybee *Apis mellifera* (Hymenoptera, Apidae). The control of this mite infestation is obtained by the use of several acaricides. Therefore, the aim of this study is to evaluate the secondary effects of the acaricidal treatments on the *Varroa* host, the honeybee, by measuring acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) activities in the larvae, pupae and adult stages of *A. mellifera intermissa*. Four groups of five hives each were treated with acaricides (flumethrin, amitraz, thymol and thymol blended with essential oils) and one group was left untreated as control. Data showed that all acaricides have no significant effect on AChE activity. However, they led to increase GST activity in the larval instars, pupae, emerged and nurse bees, as compared to controls. In the forager bees, the GSTs activity was similar in all groups of honeybees. Bees are exposed to toxic stress when acaricides, especially synthetic ones, are used as treatments in hives.

A Imdorf, S Bogdanov, V Kilchenmann, T Berger

TOXIC EFFECTS OF ESSENTIAL OILS AND SOME OF THEIR COMPONENTS ON VARROA  
DESTRUCTOR OUD AND APIS MELLIFERA L UNDER LABORATORY CONDITIONS.

Abstract

The essential oils of thyme, salvia and of hyssop, and their main components were tested on *Varroa destructor* Oud. and on honeybees in a dose-effect laboratory test. Thyme and salvia oils, as well as two types of hyssop oil (eucalyptol and pinocamphon type) showed good acaricidal efficiency of more than 80% at concentrations above 500, 300, 500 and 400 µg/l air, respectively. Only salvia oil and pinocamphon type hyssop oil were tolerated well by bees, the other two oils caused a relatively high lethality of over 20% for bees at concentrations leading to a good mite toxicity. Apart of the known potent acaricide thymol, the thyme oil components p-cymol and γ-terpinene were most toxic for *Varroa*, while well tolerated by bees at concentrations between 400 to 1000 and 350 to 800 µg/l air respectively. During the application of salvia oil, the concentration of its main components α-thujone, camphor and eucalyptol was not high enough to achieve a good acaricidal effect. On the other hand, when these three components were tested as pure substances at the proper concentrations, a high toxicity against *Varroa* was observed, while they were well tolerated by the bees at concentrations between 150 to 350, 50 to 150 and 240 to 300 µg/l air, respectively. Agroscope Liebefeld-Posieux Swiss Federal Research Station for Animal Production and Dairy Products (ALP) Swiss Bee Research Centre Schwarzenburgstrasse 161 CH-3003 Bern / Switzerland Phone +41 (0)31 323 84 18 Fax +41 (0)31 323 82 27

Boncrisiani H, Underwood R, Schwarz R, Evans JD, Pettis J, vanEngelsdorp D

J Insect Physiol. 2012 May; 58(5):613-20.

doi: 10.1016/j.jinsphys.2011.12.011. Epub 2011 Dec 28.

Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*.  
USDA-ARS Bee Research Lab, BARC-East Bldg. 476, Beltsville, MD 20705, USA.

Abstract

The effect of using acaricides to control varroa mites has long been a concern to the beekeeping industry due to unintended negative impacts on honey bee health. Irregular ontogenesis, suppression of immune defenses, and impairment of normal behavior have been linked to pesticide use. External stressors, including parasites and the pathogens they vector, can confound studies on the effects of pesticides on the metabolism of honey bees. This is the case of *Varroa destructor*, a mite that negatively affects honey bee health on many levels, from direct parasitism, which diminishes honey bee productivity, to vectoring and/or activating other pathogens, including many viruses. Here we present a gene expression profile comprising genes acting on diverse metabolic levels (detoxification, immunity, and development) in a honey bee population that lacks the influence of varroa mites. We present data for hives treated with five different acaricides; Apiguard (thymol), Apistan (tau-fluvalinate), Checkmite (coumaphos), Miteaway (formic acid) and ApiVar (amitraz). The results indicate that thymol, coumaphos and formic acid are able to alter some metabolic responses. These include detoxification gene expression pathways, components of the immune system responsible for cellular response and the c-Jun amino-terminal kinase (JNK) pathway, and developmental genes. These could potentially interfere with the health of individual honey bees and entire colonies.

Apidologie

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Exposure to thymol decreased phototactic behaviour in the honeybee (*Apis mellifera*) in laboratory conditions

Maelys Bergougnot, Michel Treilhou, Catherine Armengaud

Abstract

The effects of the terpenoid thymol were evaluated on the phototactic behaviour of the adult honeybee (*Apis mellifera*) 1 and 24 h after a topical application. The behaviour was quantified under different light intensities by measuring the time spent in the light source area and in areas opposite the source. Stimuli of 200 lx induced positive phototaxis of the bees. Thymol administered at 1 ng/bee had no effect on the phototactic behaviour while bees that had received 10 or 100 ng thymol 1 h before the test were less attracted by the 200-lx stimulus. The effect of thymol increased when the phototactic behaviour was tested 24 h after the topical application. However, with a light intensity of 400 lx the dose 10 ng/bee was ineffective and for 600 lx the phototactic behaviour of the bees was not modified by the exposure to thymol.

Environmental Science and Pollution Research

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Thymol as an alternative to pesticides: persistence and effects of Apilife Var on the phototactic behavior of the honeybee *Apis mellifera*

Jean-Luc Carayon, Nathan Téné, Elsa Bonnafé, Julie Alayrangues, Lucie Hotier, Catherine Armengaud, Michel Treilhou

Abstract

Thymol is a natural substance increasingly used as an alternative to pesticides in the fight against the *Varroa destructor* mite. Despite the effectiveness of this phenolic monoterpene against *Varroa*, few articles have covered the negative or side effects of thymol on bees. In a previous study, we have found an impairment of phototaxis in honeybees following application of sublethal doses of thymol—lower or equal to 100 ng/bee—under laboratory conditions. The present work shows the same behavioral effects on bees from hives treated with Apilife Var®, a veterinary drug containing 74 % thymol, with a decrease in phototactic behavior observed 1 day after treatment. Thus, thymol causes disruption of bee phototactic behavior both under laboratory conditions as well as in beehives. The bee exposure dose in treated hives was quantified using gas chromatography coupled to mass spectrometry (GC–MS), giving a median value of 4.3 µg per body 24 h after treatment, with 11 ng in the brain. The thymol level in 20 organic waxes from hives treated with Apilife Var® was also measured and showed that it persists in waxes (around 10 mg/kg) 1 year after treatment. Thus, in the light of (1) behavioral data obtained under laboratory conditions and in beehives, (2) the persistence of thymol in waxes, and (3) the high load on bees, it would appear important to study the long-term effects of thymol in beehives.

Environmental Science and Pollution Research

March 2014 (ePub ahead of print)

Effect of a thymol application on olfactory memory and gene expression levels in the brain of the honeybee *Apis mellifera*

Elsa Bonnafé, Florian Drouard, Lucie Hotier, Jean-Luc Carayon, Pierre Marty, Michel Treilhou, Catherine Armengaud

#### Abstract

Essential oils are used by beekeepers to control the Varroa mites that infest honeybee colonies. So, bees can be exposed to thymol formulations in the hive. The effects of the monoterpene thymol were explored on olfactory memory and gene expression in the brain of the honeybee. In bees previously exposed to thymol (10 or 100 ng/bee), the specificity of the response to the conditioned stimulus (CS) was lost 24 h after learning. Besides, the octopamine receptor OAR1 gene *Amoa1* showed a significant decrease of expression 3 h after exposure with 10 or 100 ng/bee of thymol. With the same doses, expression of *Rdl* gene, coding for a GABA receptor subunit, was not significantly modified but the *trpl* gene was upregulated 1 and 24 h after exposure to thymol. These data indicated that the genes coding for the cellular targets of thymol could be rapidly regulated after exposure to this molecule. Memory and sensory processes should be investigated in bees after chronic exposure in the hive to thymol-based preparations.

Lethal and sub-lethal effects of thymol on honeybee (*Apis mellifera*) larvae reared in vitro

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b Department of Evolution and Biological Diversity, University of Toulouse, France

#### Abstract

**BACKGROUND:** Thymol offers an attractive alternative to synthetic chemicals to keep Varroa under control. However, thymol accumulates in bee products and is suspected of having adverse effects on colonies and especially on larvae. In this study, we investigated the effects of acute and chronic exposure to thymol on larvae reared in vitro with contaminated food and compared results to the theoretical larval exposure based on the amount of pollen and honey consumed by larvae during their development.

**RESULTS:** The laboratory assays reveal that, first, the 48 h-LD<sub>50</sub> of thymol introduced into larval food is 0.044 mg larva<sup>-1</sup>. Second, the 6 day-LC<sub>50</sub> is 700 mg kg<sup>-1</sup> food. A significant decrease of larval survival and mass occurred from 500 mg thymol kg<sup>-1</sup> food (P<0.0001). Finally, vitellogenin expression, which reached a maximum at the fifth instar larvae, is delayed for individuals exposed to 50 mg thymol kg<sup>-1</sup> food (P<0.0006). That is 10 times higher than the theoretical level of exposure.

**CONCLUSION:** Based on the level of thymol residue found in honey and pollen, these results suggest that the contamination of food by thymol represents no notable risk for the early-developing larvae.

*Pest Manag Sci* 2014;70: 140–147

<http://www.mdpi.com/2075-4450/8/3/84/pdf>

Effects of Oxalic Acid on *Apis mellifera* (Hymenoptera: Apidae) Eva Rademacher, Marika Harz and Saskia Schneider

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*Insects* 2017, 8, 84; doi:10.3390/insects8030084

Oxalic acid dihydrate is used to treat varroosis of *Apis mellifera*. This study investigates lethal and sublethal effects of oxalic acid dihydrate on individually treated honeybees kept in cages under laboratory conditions as well as the distribution in the colony. After oral application, bee mortality occurred at relatively low concentrations (No Observed Adverse Effect Level (NOAEL) 50 µg/bee; Lowest Observed Adverse Effect Level (LOAEL) 75 µg/bee) compared to the dermal treatment (NOAEL 212.5 µg/bee; LOAEL 250 µg/bee). The dosage used in regular treatment via dermal application (circa 175 µg/bee) is below the LOAEL, referring to mortality derived in the laboratory. However, the treatment with oxalic acid dihydrate caused sublethal effects: This could be demonstrated in an increased responsiveness to water, decreased longevity and a reduction in pH-values in the digestive system and the hemolymph. The shift towards stronger acidity after treatment confirms that damage to the epithelial tissue and organs is likely to be caused by hyperacidity. The distribution of oxalic acid dihydrate within a colony was shown by macro-computed tomography; it was rapid and consistent. The increased density of the individual

bee was continuous for at least 14 days after the treatment indicating the presence of oxalic acid dihydrate in the hive even long after a treatment.

## EFFECT OF SUB-LETHAL DOSES OF SYNTHETIC AND NATURAL ACARICIDES ON HONEY BEE (*Apis mellifera* L.) HEALTH, MEMORY, BEHAVIOUR AND ASSOCIATED GENE EXPRESSION

Gashout, Hanan (2017)

[https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/10230/Gashout\\_Hanan\\_201701\\_PhD.pdf?sequence=1&isAllowed=y](https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/10230/Gashout_Hanan_201701_PhD.pdf?sequence=1&isAllowed=y)

Acaricides are applied in honey bee (*Apis mellifera* L.) colonies to control parasitic mites, including *Varroa destructor*. However, acaricides may cause adverse effects to honey bees. In three studies here reported, bees were exposed to lethal and sublethal doses of five of the most commonly used hive acaricides, and their impact on honey bee health, memory and behaviour, as well as on the immune and neural systems of bees was evaluated. Calculated LD50s showed that tau-fluvalinate is the most toxic among the tested acaricides (0.45 µg/bee), followed by amitraz (1.99 µg/bee), coumaphos (6.23 µg/bee), thymol (51.25 µg/bee) and formic acid (152.45 µg/bee). In term of hazard ratios (estimates of hive level risk), tau-fluvalinate was the most hazardous acaricide (3.6), followed by formic acid (0.9), and then amitraz, coumaphos and thymol, with the same hazard ratio (0.5). The expression of health and detoxification genes was analyzed and it was found that vitellogenin was not affected by any of the acaricides and that formic acid was the only acaricide that down-regulated cytochrome P450, suggesting that detoxification mechanisms of the exposed bees were in part compromised. Conversely, all acaricides except tau-fluvalinate increased the expression of the gene encoding acetylcholinesterase. The effect of sublethal doses (LD05) of acaricides on memory was evaluated. Formic acid significantly affected the bees' short, mid and long-term memory, while the rest of the acaricides affected memory only in the long term. Memory related genes were analyzed and it was found that neurexin, neuroligin-1 and major royal jelly protein-1 were not affected by any of the acaricides at sublethal doses. However, thymol, formic acid, amitraz and coumaphos increased defensin-1 expression, suggesting induction of immune responses, which also could have detrimental effects in bees' brains. The hygienic behaviour of bees was inhibited only when exposed to sublethal doses of coumaphos but not to the other acaricides, whereas tau-fluvalinate, coumaphos and formic acid reduced their foraging behaviour. Overall, it can be concluded that sub-lethal exposure to acaricides affects the health, memory and behaviour of honey bees differently, and that amitraz and thymol, appear to be the safest acaricides to bees among the five tested.

## Feral nest structure and criteria for nest selection by swarms

The nest of the honey bee (*Apis mellifera* L.)

Journal *Insectes Sociaux* Volume 23, Number 4 / December, 1976, Pages 495-512

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### Summary

The natural honey bee nest was studied in detail to better understand the honey bee's natural living conditions. To describe the nest site we made external observations on 39 nests in hollow trees. We collected and dissected 21 of these tree nests to describe the nest architecture. No one tree genus strongly predominates among bee trees. Nest cavities are vertically elongate and approximately cylindrical. Most are 30 to 60 liters in volume and at the base of trees. Nest entrances tend to be small, 10 to 40 cm<sup>2</sup>, and at the nest bottom. Rough bark outside the entrance is often smoothed by the bees. Inside the nest, a thin layer of hardened plant resins (propolis) coats the cavity walls. Combs are fastened to the walls along their tops and sides, but bees leave small passageways along the comb edges. The basic nest organization is honey storage above, brood nest below, and pollen storage in between. Associated with this arrangement are differences in comb structure. Compared to combs used for honey storage, combs of the brood nest are generally darker and more uniform in width and in cell form. Drone comb is located on the brood nest's periphery. Comparisons among *Apis* nests indicate the advanced characters in *Apis mellifera* nests arose in response to *Apis mellifera*'s adoption of tree cavities for nest sites.



Nest site selection by the honey bee, *Apis mellifera*

Insectes Sociaux

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Received: 22 November 1977 Accepted: 9 March 1978

**Summary** 1. Honey bees exhibit preferences in several nest site properties. The following preferences were identified («>» means «preferred to»): nest height, 5>1 m; entrance area, 12.5>75 cm<sup>2</sup>; entrance position, bottom > top of nest cavity, entrance direction, southward > northward; nest cavity volume, 10<40>100 liters.

2. The data also suggest preferences exist for previously inhabited nest cavities and for nest sites beyond 300 m from the parent colony.

3. Nest sites with high exposure and visibility were occupied more rapidly than sites with low exposure and visibility. However, this difference probably reflects differential ease of nest site discovery rather than a preference for exposed nest sites.

4. No preferences were found in the following variables: entrance shape (slit vs. circle), nest cavity shape (cube vs. tall parallelepiped), cavity draftiness (sound vs. drafty), and cavity dryness (wet vs. dry). Cavity draftiness and dryness are probably important to bees, but because bees can seal and waterproof their nests, they may be less demanding about these two nest site variables than about those they cannot modify.

5. The complex process of nest site selection apparently benefits a honey bee colony in several ways, including facilitation of colony defense and hygiene, simplification of nest construction and microclimate control, and reduction of foraging competition with the parent colony.

## **Advantage of intracolony genetic diversity obtained through plentiful drone populations enabling adequate multiple mating and consequent fitness**

Regarding the following paper Neumann & Blacquière (2016) comment: "A recent study showed that honey bee colonies, which were made hyper- polyandrous artificially (30 or 60 matings), had improved performance (Delaplane et al. (2015), thereby suggesting that genetic diversity of *A. mellifera* has already been lost and thus drone mates may be too genetically similar by now."

Honey Bee Colonies Headed by Hyperpolyandrous Queens Have Improved Brood Rearing Efficiency and Lower Infestation Rates of Parasitic *Varroa* Mites

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PLoS ONE, 10(12), e0142985. doi:10.1371/journal.pone.0142985

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**Abstract** A honey bee queen mates on wing with an average of 12 males and stores their sperm to produce progeny of mixed paternity. The degree of a queen's polyandry is positively associated with measures of her colony's fitness, and observed distributions of mating number are evolutionary optima balancing risks of mating flights against benefits to the colony. Effective mating numbers as high as 40 have been documented, begging the question of the upper bounds of this behavior that can be expected to confer colony benefit. In this study we used instrumental insemination to create three classes of queens with exaggerated range of polyandry—15, 30, or 60 drones. Colonies headed by queens inseminated with 30 or 60 drones produced more brood per bee and had a lower proportion of samples positive for *Varroa destructor* mites than colonies whose queens were inseminated with 15 drones, suggesting benefits of polyandry at

rates higher than those normally obtaining in nature. Our results are consistent with two hypotheses that posit conditions that reward such high expressions of polyandry: (1) a queen may mate with many males in order to promote beneficial non-additive genetic interactions among subfamilies, and (2) a queen may mate with many males in order to capture a large number of rare alleles that regulate resistance to pathogens and parasites in a breeding population. Our results are unique for identifying the highest levels of polyandry yet detected that confer colony-level benefit and for showing a benefit of polyandry in particular toward the parasitic mite *V. destructor*.

Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth

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Multiple mating by social insect queens increases the genetic diversity among colony members, thereby reducing intracolony relatedness and lowering the potential inclusive fitness gains of altruistic workers. Increased genetic diversity may be adaptive, however, by reducing the prevalence of disease within a nest. Honeybees, whose queens have the highest levels of multiple mating among social insects, were investigated to determine whether genetic variation helps to prevent chronic infections. I instrumentally inseminated honeybee queens with semen that was either genetically similar (from one male) or genetically diverse (from multiple males), and then inoculated their colonies with spores of *Ascosphaera apis*, a fungal pathogen that kills developing brood. I show that genetically diverse colonies had a lower variance in disease prevalence than genetically similar colonies, which suggests that genetic diversity may benefit colonies by preventing severe infections.

Proc. R. Soc. Lond. B (2003) 270, 99–103 © 2002 The Royal Society

DOI 10.1098/rspb.2002.2199

Naturwissenschaften (2006) 93: 195–199

David R. Tarpy, Thomas D. Seeley

Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens

Abstract

We studied the relationship between genetic diversity and disease susceptibility in honeybee colonies living under natural conditions. To do so, we created colonies in which each queen was artificially inseminated with sperm from either one or ten drones. Of the 20 colonies studied, 80% showed at least one brood disease. We found strong differences between the two types of colonies in the infection intensity of chalkbrood and in the total intensity of all brood diseases (chalkbrood, sacbrood, American foulbrood, and European foulbrood) with both variables lower for the colonies with higher genetic diversity. Our findings demonstrate that disease can be an important factor in the ecology of honeybee colonies and they provide strong support for the disease hypothesis for the evolution of polyandry by social insect queens.

Proc. R. Soc. B (2007) 274, 67–72

doi:10.1098/rspb.2006.3702

Published online 26 September 2006

Queen promiscuity lowers disease within honeybee colonies

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Most species of social insects have singly mated queens, but in some species each queen mates with numerous males to create a colony with a genetically diverse worker force. The adaptive significance of polyandry by social insect queens remains an evolutionary puzzle. Using the honeybee (*Apis mellifera*), we tested the hypothesis that polyandry improves a colony's resistance to disease. We established colonies headed by queens that had been artificially inseminated by either one or 10 drones. Later, we inoculated these colonies with spores of *Paenibacillus larvae*, the bacterium that causes a highly virulent disease of honeybee larvae (American foulbrood). We found that, on average, colonies headed by multiple-drone inseminated queens had markedly lower disease intensity and higher colony strength at the end of the summer relative to colonies headed by single-drone inseminated queens. These findings support the

hypothesis that polyandry by social insect queens is an adaptation to counter disease within their colonies.

Proc. R. Soc. Lond. B (1998) 265, 2009-2014

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Relatedness among honeybees (*Apis mellifera*) of a drone congregation

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The honeybee (*Apis mellifera*) queen mates during nuptial flights, in the so-called drone congregation area where many males from surrounding colonies gather. Using 20 highly polymorphic microsatellite loci, we studied a sample of 142 drones captured in a congregation close to Oberursel (Germany). A parentage test based on lod score showed that this sample contained one group of four brothers, six groups of three brothers, 20 groups of two brothers and 80 singletons. These values are very close to a Poisson distribution. Therefore, colonies were apparently equally represented in the drone congregation, and calculations showed that the congregation comprised males that originated from about 240 different colonies. This figure is surprisingly high. Considering the density of colonies around the congregation area and the average flight range of males, it suggests that most colonies within the recruitment perimeter delegated drones to the congregation with an equal probability, resulting in an almost perfect panmixis. Consequently, the relatedness between a queen and her mates, and hence the inbreeding coefficient of the progeny, should be minimized. The relatedness among the drones mated to the same queen is also very low, maximizing the genetic diversity among the different patriline of a colony.

Genetic Diversity in Honey Bee Colonies Enhances Productivity and Fitness

Heather R. Mattila\* and Thomas D. Seeley

DOI: 10.1126/science.1143046

Science, 317 pp362-364 (2007); 317

Honey bee queens mate with many males, creating numerous patrilines within colonies that are genetically distinct. The effects of genetic diversity on colony productivity and long-term fitness are unknown. We show that swarms from genetically diverse colonies (15 patrilines per colony) founded new colonies faster than swarms from genetically uniform colonies (1 patriline per colony). Accumulated differences in foraging rates, food storage, and population growth led to impressive boosts in the fitness (i.e., drone production and winter survival) of genetically diverse colonies. These results further our understanding of the origins of polyandry in honey bees and its benefits for colony performance.

Proc. R. Soc. B(2008)275, 809–816

doi:10.1098/rspb.2007.1620

Published online 15 January 2008

Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers

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Recent work has demonstrated considerable benefits of intracolony genetic diversity for the productivity of honeybee colonies: single-patriline colonies have depressed foraging rates, smaller food stores and slower weight gain relative to multiple-patriline colonies. We explored whether differences in the use of foraging-related communication behaviour (waggle dances and shaking signals) underlie differences in foraging effort of genetically diverse and genetically uniform colonies. We created three pairs of colonies; each pair had one colony headed by a multiply mated queen (inseminated by 15 drones) and one colony headed by a singly mated queen. For each pair, we monitored the production of foraging-related signals over the course of 3 days. Foragers in genetically diverse colonies had substantially more information available to them about food resources than foragers in uniform colonies. On average, in genetically diverse

colonies compared with genetically uniform colonies, 36% more waggle dances were identified daily, dancers performed 62% more waggle runs per dance, foragers reported food discoveries that were farther from the nest and 91% more shaking signals were exchanged among workers each morning prior to foraging. Extreme polyandry by honeybee queens enhances the production of worker-worker communication signals that facilitate the swift discovery and exploitation of food resources.

*J. EVOL. BIOL.* 16 (2003) 914–920

Male fitness of honeybee colonies (*Apis mellifera* L.)

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Kraus, F. B., Neumann, P., Scharpenberg, H., Van Praagh, J. & Moritz, R. F. A. (2003) Male fitness of honeybee colonies (*Apis mellifera* L.). *J. Evol. Biol.* **16**, 914–920

Abstract

Honeybees (*Apis mellifera* L.) have an extreme polyandrous mating system. Worker offspring of 19 naturally mated queens was genotyped with DNA microsatellites, to estimate male reproductive success of 16 drone producing colonies. This allowed for estimating the male mating success on both the colony level and the level of individual drones. The experiment was conducted in a closed population on an isolated island to exclude interferences of drones from unknown colonies. Although all colonies had produced similar numbers of drones, differences among the colonies in male mating success exceeded one order of magnitude. These differences were enhanced by the siring success of individual drones within the offspring of mated queens. The siring success of individual drones was correlated with the mating frequency at the colony level. Thus more successful colonies not only produced drones with a higher chance of mating, but also with a significantly higher proportion of offspring sired than drones from less successful colonies. Although the life cycle of honeybee colonies is very female centred, the male reproductive success appears to be a major driver of natural selection in honeybees.

*Naturwissenschaften* (2003) 90:265–268

DOI 10.1007/s00114-003-0418-3

SHORT COMMUNICATION

Kellie A. Palmer · Benjamin P. Oldroyd

Evidence for intra-colonial genetic variance in resistance

to American foulbrood of honey bees (*Apis mellifera*): further support

for the parasite/pathogen hypothesis for the evolution of polyandry

Received: 25 July 2002 / Accepted: 23 March 2003 / Published online: 7 May 2003

Springer-Verlag 2003

Abstract:

Explanations for the evolution of multiple mating by social insect (particularly honey bee) queens have been frequently sought. An important hypothesis is that multiple mating is adaptive because it increases intracolony genetic diversity and thereby reduces the likelihood that parasites or pathogens will catastrophically infect a colony. We tested one assumption of this model: that honey bee worker patriline should differ in disease resistance. We used American foulbrood (caused by the bacterium *Paenibacillus larvae*) as a model pathogen. We found that patriline within colonies do indeed vary in their resistance to this disease.

JONES, J C; MYERSCOUGH, M R; GRAHAM, S; OLDROYD, B P (2004)

Honey bee nest thermoregulation: Diversity promotes stability .

*Science* 305: 402-404

Simulation studies of the task threshold model for task allocation in social insect colonies suggest that nest temperature homeostasis is enhanced if workers have slightly different thresholds for engaging in tasks related to nest thermoregulation. Genetic variance in task thresholds is one way a distribution of task thresholds can be generated. *Apis mellifera* colonies with large genetic diversity are able to maintain more stable brood nest temperatures than colonies that are genetically uniform. If this phenomenon is generalizable to other species, we would predict that patriline should vary in the threshold in which they engage in thermoregulatory tasks. We exposed *A. florea* colonies to different temperatures experimentally,

and retrieved fanning workers at these different temperatures. In many cases we found statistically significant differences in the proportion of fanning workers of different patrilineages at different experimental temperatures. This suggests that genetically different workers have different thresholds for performing the thermoregulatory task of fanning. We suggest, therefore, that genetically based variance in task threshold is a widespread phenomenon in the genus *Apis*.

The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth

J. Rangel, J. J. Keller, D. R. Tarpy

*Insectes Sociaux* February 2013, Volume 60, Issue 1, pp 65-73

Abstract

Reproduction in species of eusocial insects is monopolized by one or a few individuals, while the remaining colony tasks are performed by the worker caste. This reproductive division of labor is exemplified by honey bees (*Apis mellifera* L.), in which a single, polyandrous queen is the sole colony member that lays fertilized eggs. Previous work has revealed that the developmental fate of honey bee queens is highly plastic, with queens raised from younger worker larvae exhibiting higher measures in several aspects of reproductive potential compared to queens raised from older worker larvae. Here, we investigated the effects of queen reproductive potential (“quality”) on the growth and winter survival of newly established honey bee colonies. We did so by comparing the growth of colonies headed by “high-quality” queens (i.e., those raised from young worker larvae, which are more queen-like morphologically) to those headed by “low-quality” queens (i.e., those raised from older worker larvae, which are more worker-like morphologically). We confirmed that queens reared from young worker larvae were significantly larger in size than queens reared from old worker larvae. We also found a significant positive effect of queen grafting age on a colony’s production of worker comb, drone comb, and stored food (honey and pollen), although we did not find a statistically significant difference in the production of worker and drone brood, worker population, and colony weight. Our results provide evidence that in honey bees, queen developmental plasticity influences several important measures of colony fitness. Thus, the present study supports the idea that a honey bee colony can be viewed (at least in part) as the expanded phenotype of its queen, and thus selection acting predominantly at the colony level can be congruent with that at the individual level.

<http://link.springer.com/article/10.1007/s00040-012-0267-1>

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*Science* 16 July 2004:

Vol. 305. no. 5682, pp. 402 - 404

DOI: 10.1126/science.1096340

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Reports

Honey Bee Nest Thermoregulation: Diversity Promotes Stability

Julia C. Jones,<sup>1\*</sup> Mary R. Myerscough,<sup>2</sup> Sonia Graham,<sup>2</sup> Benjamin P. Oldroyd<sup>1</sup>

A honey bee colony is characterized by high genetic diversity among its workers, generated by high levels of multiple mating by its queen. Few clear benefits of this genetic diversity are known. Here we show that brood nest temperatures in genetically diverse colonies (i.e., those sired by several males) tend to be more stable than in genetically uniform ones (i.e., those sired by one male). One reason this increased stability arises is because genetically determined diversity in workers' temperature response thresholds modulates the hive-ventilating behavior of individual workers, preventing excessive colony-level responses to temperature fluctuations.

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GRAHAM, S; MYERSCOUGH, M R; JONES, J C; OLDROYD, B P (2006)

Modelling the role of intracolony genetic diversity on regulation

of brood temperature in honey bee (*Apis mellifera* L.) colonies.

*Insectes Sociaux* 53:226-232.

Graham, S., Myerscough, M. R., Jones, J. C. & Oldroyd, B. P. (2006) Modelling the role of intracolony genetic diversity on regulation of brood temperature in honey bee (*Apis mellifera* L.) colonies. *Insectes Sociaux* **53** 226-232.

**Abstract.** In polyandrous social insects such as honey bees, a worker's affinity for a particular task may be genetically influenced and so some patriline may have lower stimulus thresholds for commencing a task than others. We used simulation models to investigate the effects of intracolony diversity in the task thresholds that stimulate workers to engage in heating and cooling during nest thermoregulation. First, we simulated colonies comprised of one or 15 patrilines that were engaged in heating the brood nest, and observed that single patriline colonies maintained, on average, less stable brood nest temperatures than multiple patriline colonies. Second we simulated colonies with five patrilines that were engaged in cooling their nest, recording the proportions of bees of different patrilines that engaged in nest cooling in response to changing temperatures. Both of our simulations show remarkably similar qualitative patterns to those that we have previously observed empirically. This provides further support for the hypothesis that genetically based variability in task thresholds among patrilines within honey bee colonies is an important contributor to the ability of colonies to precisely thermoregulate their nests, and we suggest that diversity is important for optimal expression of a range of other colony-level phenotypes.

Genetic diversity promotes homeostasis in insect colonies

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<sup>2</sup> School of Life Sciences and Center for Social Dynamics and Complexity, Arizona State University, Tempe, AZ 85287-4501, USA

Oldroyd, B. P. & Fewell, J. H. (2007) Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecol. Evol.* 22(8) 408-413.

**Abstract**

Although most insect colonies are headed by a singly mated queen, some ant, wasp and bee taxa have evolved high levels of multiple mating or 'polyandry'. We argue here that a contributing factor towards the evolution of polyandry is that the resulting genetic diversity within colonies provides them with a system of genetically based task specialization, enabling them to respond resiliently to environmental perturbation. An alternate view is that genetic contributions to task specialization are a side effect of multiple mating, which evolved through other causes, and that genetically based task specialization now makes little or no contribution to colony fitness.

TRENDS in Ecology and Evolution Vol.22 No.8

*Behav Ecol Sociobiol* (2011) 65:1037–1044

Intracolony genetic diversity in honeybee (*Apis mellifera*) colonies increases pollen foraging efficiency

Bruce J. Eckholm & Kirk E. Anderson & Milagra Weiss & Gloria DeGrandi-Hoffman

**Abstract**

Multiple mating by honeybee queens results in colonies of genotypically diverse workers. Recent studies have demonstrated that increased genetic diversity within a honeybee colony increases the variation in the frequency of tasks performed by workers. We show that genotypically diverse colonies, each composed of 20 subfamilies, collect more pollen than do genotypically similar colonies, each composed of a single subfamily. However, genotypically similar colonies collect greater varieties of pollen than do genotypically diverse colonies. Further, the composition of collected pollen types is less similar among genotypically similar colonies than among genotypically diverse colonies. The response threshold model predicts that genotypic subsets of workers vary in their response to task stimuli. Consistent with this model, our findings suggest that genotypically diverse colonies likely send out fewer numbers of foragers that independently search for pollen sources (scouts) in response to protein demand by the colony, resulting in a lower variety of collected pollen types. The cooperative foraging strategy of honeybees involves a limited number of scouts monitoring the environment that then guide the majority of foragers to high quality food sources. The genetic composition of the colony appears to play an important role in the efficiency of this behavior.

Citation: Mattila HR, Rios D, Walker-Sperling VE, Roeselers G, Newton ILG (2012)

Characterization of the Active Microbiotas Associated with Honey Bees Reveals Healthier and Broader Communities when Colonies are Genetically Diverse.

*PLoS ONE* 7(3): e32962. doi:10.1371/journal.pone.0032962

## Abstract

Recent losses of honey bee colonies have led to increased interest in the microbial communities that are associated with these important pollinators. A critical function that bacteria perform for their honey bee hosts, but one that is poorly understood, is the transformation of worker-collected pollen into bee bread, a nutritious food product that can be stored for long periods in colonies. We used 16S rRNA pyrosequencing to comprehensively characterize in genetically diverse and genetically uniform colonies the active bacterial communities that are found on honey bees, in their digestive tracts, and in bee bread. This method provided insights that have not been revealed by past studies into the content and benefits of honey bee-associated microbial communities. Colony microbiotas differed substantially between sampling environments and were dominated by several anaerobic bacterial genera never before associated with honey bees, but renowned for their use by humans to ferment food. Colonies with genetically diverse populations of workers, a result of the highly promiscuous mating behavior of queens, benefited from greater microbial diversity, reduced pathogen loads, and increased abundance of putatively helpful bacteria, particularly species from the potentially probiotic genus *Bifidobacterium*. Across all colonies, *Bifidobacterium* activity was negatively correlated with the activity of genera that include pathogenic microbes; this relationship suggests a possible target for understanding whether microbes provide protective benefits to honey bees. Within-colony diversity shapes microbiotas associated with honey bees in ways that may have important repercussions for colony function and health. Our findings illuminate the importance of honey bee-bacteria symbioses and examine their intersection with nutrition, pathogen load, and genetic diversity, factors that are considered key to understanding honey bee decline.

## Apidologie

May 2014, Volume 45, Issue 3, pp 347-363

Date: 21 Nov 2013

Extreme polyandry improves a honey bee colony's ability to track dynamic foraging opportunities via greater activity of inspecting bees

Heather R. Mattila, Thomas D. Seeley

## Abstract

We explored the worker-level interactions that enhance the organization of foraging in honey bee colonies with extremely polyandrous queens by determining whether a colony's patriline number affects the activity of its inspectors (foragers who visit a previously utilized food source to see if it is profitable again). We monitored the use of sucrose feeders by foragers in free-flying colonies that had either multiple patrilines or a single patriline as the feeders were sequentially stocked, emptied, and then restocked. Multiple-patriline colonies tended to have more inspectors than single-patriline colonies, their inspectors inspected their feeder at higher rates when it was empty, and then foraged at higher rates and performed more waggle runs when it was restocked, which quadrupled feeder recruitment. The patriline profile for a colony's inspectors consistently differed from that of its general population. We show clear ergonomic benefits of extreme polyandry for honey bee queens and their colonies.

Impact of Food Availability, Pathogen Exposure, and Genetic Diversity on Thermoregulation in Honey Bees (*Apis mellifera*)

M. Simone-Finstrom & B. Foo & D. R. Tarpy &

P. T. Starks

Revised: 27 February 2014 / Accepted: 4 March 2014 /

Published online: 20 March 2014

#Springer Science+Business Media New York 2014

## Abstract

Accurate thermoregulation in honey bees is crucial for colony survival. Multiple factors influence how colonies manage in-hive temperature, including genetic diversity. We explored the influence of genetic diversity on thermoregulatory behavior under three conditions: natural foraging, supplemental feeding, and exposure to the fungal pathogen shown to induce a social fever in honey bees. Our data suggest that (1) the degree of genetic diversity expected under normal conditions is not predictive of thermoregulatory stability, (2) the social fever response of honey bees is not a simple stimulus-response mechanism but appears to be influenced by ambient temperature conditions, and (3) a temperature-based circadian rhythm emerges under

high nectar flow conditions. Taken together, these data suggest that a richer, context-dependent thermoregulatory system exists in honey bees than previously understood.

J Insect Behav (2014) 27:527–539 DOI 10.1007/s10905-014-9447-3

Genetic diversity affects colony survivorship in commercial honey bee colonies

David R. Tarpy & Dennis vanEngelsdorp & Jeffrey S. Pettis

Abstract

Honey bee (*Apis mellifera*) queens mate with unusually high numbers of males (average of approximately 12 drones), although there is much variation among queens. One main consequence of such extreme polyandry is an increased diversity of worker genotypes within a colony, which has been shown empirically to confer significant adaptive advantages that result in higher colony productivity and survival. Moreover, honey bees are the primary insect pollinators used in modern commercial production agriculture, and their populations have been in decline worldwide. Here, we compare the mating frequencies of queens, and therefore, intracolony genetic diversity, in three commercial beekeeping operations to determine how they correlate with various measures of colony health and productivity, particularly the likelihood of queen supersession and colony survival in functional, intensively managed beehives. We found the average effective paternity frequency ( $m_e$ ) of this population of honey bee queens to be  $13.6 \pm 6.76$ , which was not significantly different between colonies that superseded their queen and those that did not. However, colonies that were less genetically diverse (headed by queens with  $m_e \leq 7.0$ ) were 2.86 times more likely to die by the end of the study when compared to colonies that were more genetically diverse (headed by queens with  $m_e > 7.0$ ). The stark contrast in colony survival based on increased genetic diversity suggests that there are important tangible benefits of increased queen mating number in managed honey bees, although the exact mechanism(s) that govern these benefits have not been fully elucidated.

Naturwissenschaften (2013) 100:723–728

DOI 10.1007/s00114-013-1065-y

Behavioral Ecology and Sociobiology

Date: 15 Jul 2015

Genetic diversity within honey bee colonies affects pathogen load and relative virus levels in honey bees, *Apis mellifera* L

Suresh D. Desai, Robert W. Currie

The evolution of polyandry is one of most highly debated topics in sociobiology. One hypothesis suggests genetic diversity increases resistance against the wide range of parasites and diseases affecting colonies. We investigated effects of manipulating genetic diversity on colony population size, fall *Varroa* population, *Varroa* and bee mortality rates, virus prevalence and concentration, and prevalence of *Nosema apis* Z. and *Nosema ceranae* F. Sister queens selected for resistance to *Varroa* were inseminated with either mixed semen from 12 drone sources (genetically diverse colonies, GDC) or single drone inseminated from 12 drone sources (genetically similar colonies, GSC), and colonies from these queens were compared to unrelated queens that were open-mated. When exposed to parasites and pathogens, open-mated colonies (OMC) and GDCs had larger worker populations before winter than GSCs. The bees of GDCs had higher *Varroa* mortality rates than those of GSCs in the field study. Within the instrumentally inseminated treatments, three pathogens occurred at lower prevalence (acute bee paralysis virus (ABPV) and *N. ceranae*) or concentrations (deformed wing virus, DWV) in GDCs than in GSCs. Both GDCs and GSCs that had been selected for resistance to *Varroa* had lower DWV concentration in spring when compared to unselected OMCs. Colonies inseminated by multiple drones and having greater within-colony genetic diversity showed reduced prevalence, concentrations in about a quarter of the pathogens assayed relative to genetically uniform colonies, and the reverse pattern was never observed.

**COMMENT: The following paper serves to remind of the potential genetic diversity available during open mating in drone congregation areas.**

Proc Biol Sci. 1998 Oct 22; 265(1409): 2009–2014.

doi: 10.1098/rspb.1998.0533

PMCID: PMC1689479

Relatedness among honeybees (*Apis mellifera*) of a drone congregation



E. Baudry, M. Solignac, L. Garnery, M. Gries, J.-M. Cornuet, and N. Koeniger

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Abstract

The honeybee (*Apis mellifera*) queen mates during nuptial flights, in the so-called drone congregation area where many males from surrounding colonies gather. Using 20 highly polymorphic microsatellite loci, we studied a sample of 142 drones captured in a congregation close to Oberursel (Germany). A parentage test based on lod score showed that this sample contained one group of four brothers, six groups of three brothers, 20 groups of two brothers and 80 singletons. These values are very close to a Poisson distribution. Therefore, colonies were apparently equally represented in the drone congregation, and calculations showed that the congregation comprised males that originated from about 240 different colonies. This figure is surprisingly high. Considering the density of colonies around the congregation area and the average flight range of males, it suggests that most colonies within the recruitment perimeter delegated drones to the congregation with an equal probability, resulting in an almost perfect panmixis. Consequently, the relatedness between a queen and her mates, and hence the inbreeding coefficient of the progeny, should be minimized. The relatedness among the drones mated to the same queen is also very low, maximizing the genetic diversity among the different patriline of a colony.

## Role of drones in nest thermoregulation

**COMMENT: Drone culling and the exclusive use of worker foundation may limit drone populations in colonies to below natural levels.**

Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology

Volume 159, Number 5 (1989), 551-560, DOI: 10.1007/BF00694379

Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes

L. Fahrenholz, I. Lamprecht and B. Schrickler

Abstract

The temperature at the centre, the periphery and the entrance of a honey bee colony (*Apis mellifera carnica*) was continuously determined during the summer season and the broodless time in winter. During the summer season the temperature in the brood nest averages 35.5°C with brief excursions up to 37.0°C and down to 33.8°C. Increasing environmental temperatures resulted in linear increases in the temperature of the hive entrance, its periphery and its centre. The temperature in the centre of an overwintering cluster is maintained at an average value of 21.3°C (min 12.0°C, max 33.5°C). With rising ambient temperatures the central temperature of a winter cluster drops whereas the peripheral temperature increases slightly. With decreasing external temperatures the peripheral temperature is lowered by a small amount while the cluster's centre temperature is raised. Linear relationships are observed between the central and the ambient temperature and between the central temperature and the temperature difference of the peripheral and the ambient temperatures. The slopes point to two minimum threshold values for the central (15°C) and the peripheral temperature (5°C) which should not be transgressed in an overwintering cluster.

Microcalorimetric determinations of the heat production were performed on the three castes of the honey bee: workers, drones and queens of different ages. Among these groups single adult workers showed the highest heat production rates (209 mW·g<sup>-1</sup>) with only neglectible fluctuations in the heat production rate. Juvenile workers exhibited a mean heat production rate of 142 mW·g<sup>-1</sup>. The rate of heat production of adult workers is strongly dependent upon the number of bees together in a group. With more than 10 individuals weight-specific heat dissipation remains constant with increasing group sizes at a level approximately 1/17 that of an isolated bee. Differences are seen between the rates of virgin (117 mW·g<sup>-1</sup>) and laying (102 mW·g<sup>-1</sup>) queens. Laying queens showed less thermal fluctuations than virgin queens. High fluctuations in heat production rates are observed for drones. In both groups (fertile, juvenile) phases of high and extremely low activity succeed one another. The heat production of juvenile drones was 68 mW·g<sup>-1</sup>, that of fertile drones 184 mW·g<sup>-1</sup> due to stronger locomotory activities.

Apidologie 40 (2009) 82–95 Available online at:

c 7 INRA/DIB-AGIB/EDP Sciences, 2009 www.apidologie.org

DOI:10.1051/apido/2008069

Original article

Contribution of honeybee drones of different age to colonial thermoregulation\*

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Received 8 May 2008 – Revised 23 October 2008 – Accepted 11 November 2008

**Abstract**– In addition to honeybee workers, drones also contribute to colonial thermoregulation. We show the drones' contribution to thermoregulation at 5 different experimental temperatures ranging from 15–34°C. The frequency and the degree of endothermy depended on the drones' local ambient temperature and age. Location on brood or non-brood areas had no influence. The frequency of endothermic drones and the intensity of endothermy increased with decreasing temperature. 30% of drones of 8 days and older heated their thorax by more than 1°C

above the abdomen. The youngest drones (0–2 days) did not exceed this level of endothermy. Though young drones were less often engaged in active heat production, their contribution to brood warming was not insignificant because their abundance on the brood nest was 3.5 times higher than that of the oldest drones ( $\geq 13$  days). Results suggest that the stimulus for the drones' increased frequency of heating at low experimental temperatures was their low local ambient air and/or comb temperature.

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ROLES OF INDIVIDUAL HONEYBEE WORKERS AND DRONES IN COLONIAL THERMOGENESIS  
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SUMMARY

1. The individual roles of honeybee workers and drones in heat regulation were investigated using single combs of bees and brood (about 1000 individuals) placed in boxes at 15°C. After 1 h and before cluster formation, I measured the elevation of bee thoracic surface temperature ( $T_{\text{ths}}$ ) above local ambient temperature ( $T_{\text{a}}$ ). Bees were then left overnight at 15°C.
2. During the preclustering period, the density of bees over the brood slowly increased. In the clusters left overnight, bees in the innermost layer were significantly younger than bees in the outermost layer. One-day-old bees and drones were always located in the innermost cluster layer.
3. 89% of all workers measured had  $T_{\text{ths}} - T_{\text{a}} \geq 2^\circ\text{C}$ , indicating that most workers contribute to colonial thermogenesis. Average  $T_{\text{ths}} - T_{\text{a}}$  was 4.1°C. Drones measured had the same average  $T_{\text{ths}} - T_{\text{a}}$  as unmarked workers.  $T_{\text{ths}} - T_{\text{a}}$  did not differ among bees 2 days of age and older. Location on or off the brood did not affect  $T_{\text{ths}} - T_{\text{a}}$ .
4. Cooling constants of dead bees placed near the comb in the box averaged  $1.036 \text{ min}^{-1}$  and were independent of location on the comb. Calculated average thoracic conductance was  $0.829 \text{ cal g}^{-1} \text{ }^\circ\text{C}^{-1} \text{ min}^{-1}$ .
5. Average calculated heat production per worker was  $0.095 \text{ cal min}^{-1}$ , less than 15% of the maximal oxygen consumption of 4-day-old bees. Calculations indicate that the larger drones contribute more heat per bee than do the workers.
6. Brood warming prior to clustering is due to relatively uniform heat production by individual bees and the attraction of bees to the brood area. This results in a higher heat production per unit volume of air over the brood than away from the brood.

## **Importance of species-specific behaviours, e.g. social immunity (propolis deposition), in disease resistance**

**COMMENT: Breeding for low propolis collection could be harmful**

Seasonal benefits of a natural propolis envelope to honey bee immunity and colony health

Renata S. Borba, Karen K. Klyczek, Kim L. Mogen, Marla Spivak

Journal of Experimental Biology 2015 : doi: 10.1242/jeb.127324

Honey bees, as social insects, rely on collective behavioral defenses that produce a colony level immune phenotype, or social immunity, which in turn impacts the immune response of individuals. One behavioral defense is the collection and deposition of antimicrobial plant resins, or propolis, in the nest. We tested the effect of a naturally constructed propolis envelope within standard beekeeping equipment on the pathogen and parasite load of large field colonies, and on immune system activity, virus and storage protein levels of individual bees over the course of a year. The main effect of the propolis envelope was a decreased and more uniform baseline expression of immune genes in bees during summer and fall months each year, compared to the immune activity in bees with no propolis envelope in the colony. The most important function of the propolis envelope may be to modulate costly immune system activity. As no differences were found in levels of bacteria, pathogens and parasites between the treatment groups, the propolis envelope may act directly on the immune system, reducing bees' need to activate the physiologically costly production of humoral immune responses. Colonies with a natural propolis envelope had increased colony strength and vitellogenin levels after surviving the winter in one of the two years of the study, despite the fact that the biological activity of the propolis diminished over the winter. A natural propolis envelope acts as an important antimicrobial layer enshrouding the colony, benefiting individual immunity and ultimately colony health.

<http://jeb.biologists.org/content/early/2015/10/07/jeb.127324>

Apidologie 41 (2010) 295–311

Review article

Propolis and bee health: the natural history and significance of resin use by honey bees\*

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2 Department of Entomology, University of Minnesota, Twin Cities, St. Paul, Minnesota 55108, USA

Abstract–

Social immunity, which describes how individual behaviors of group members effectively reduce disease and parasite transmission at the colony level, is an emerging field in social insect biology. An understudied, but significant behavioral disease resistance mechanism in honey bees is their collection and use of plant resins. Honey bees harvest resins with antimicrobial properties from various plant species and bring them back to the colony where they are then mixed with varying amounts of wax and utilized as propolis. Propolis is an apicultural term for the resins when used by bees within a hive. While numerous studies have investigated the chemical components of propolis that could be used to treat human diseases, there is a lack of information on the importance of propolis in regards to bee health. This review serves to provide a compilation of recent research concerning the behavior of bees in relation to resins and propolis, focusing more on the bees themselves and the potential evolutionary benefits of resin collection. Future research goals are also established in order to create a new focus within the literature on the natural history of resin use among the social insects and role that propolis plays in disease resistance.

Apidologie March 2014, Volume 45, Issue 2, pp 269-275

Increased brood viability and longer lifespan of honeybees selected for propolis production

Daniel Nicodemo, Euclides Braga Malheiros, David De Jong, Regina Helena Nogueira Couto

Abstract

Propolis has been proposed to affect honeybee health. To test this hypothesis, we initially evaluated propolis production in 36 honeybee colonies. The three highest (HP) and three lowest propolis-producing (LP) colonies had mean yields of 16.0 and 0.64 g, respectively. Queens and drones from these parental colonies were crossed by artificial insemination to produce five colonies each of the following crosses: HP♀×HP♂, HP♀×LP♂, LP♀×HP♂, and LP♀×LP♂. Colonies headed by HP♀×HP♂ queens produced 34 times more

propolis than those headed by LP♀×LP♂ queens and five times more than those from the other two crosses. Newly emerged bees were marked to measure longevity, and egg and brood counts were made to determine brood survival rates. The colonies with queens derived from crosses between high-propolis-producing colonies had significantly higher brood viability and greater worker bee longevity. **We conclude that colonies that collect more propolis are healthier and have longer-living bees.**

Pathway and transcriptional insights into honey bee immunity from the Honey Bee Genome Project

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evansj@ba.ars.usda.gov <mailto:evansj@ba.ars.usda.gov>

Abstract

Honey bees combat disease through both social, or group-level, mechanisms and individual defenses. Bees face significant parasites and pathogens across many taxonomic groups, including bacteria, viruses, protists, and fungi, and a wealth of pathology data for honey bees can be used to explore host-parasite interactions. Like other insects, honey bees use components of the innate immune response to defend against pathogens, and recent work has explored the mechanisms<sup>1</sup> and efficacy<sup>2</sup> of this response. With the sequencing, assembly, and annotation of the honey bee genome it is possible to propose and test hypothetical immune-pathway models for bees. Honey bees show likely orthologs for nearly all members of the canonical insect innate immune pathways (Toll, Imd, JNK, and Jak/STAT). Surprisingly, honey bees have sharply lower immune-pathway redundancy when compared to other insects, implying decreased flexibility in the immune responses of bees toward pathogens. Of 17 immune gene families implicated in recognition, signaling, and effecting an immune response, bees have approximately 1/3 the gene diversity found in the *Drosophila melanogaster* and *Anopheles gambiae* genomes. This reduction could reflect **the protective strength of behavioral and environmental barriers to honey bee disease**, a tendency of bees to be attacked by a limited set of coevolved pathogens, or novel, parallel mechanisms with which bees achieve immune response flexibility. Transcript abundance levels for pathway members and effectors are presented in order to describe the covariance and heritability of immune responses in bees. <sup>1</sup> K.

Aronstein, E. Saldivar,

*Apidologie*/ 36, 3--14 (2005), <sup>2</sup> J. D. Evans, J. S. Pettis, *Evolution*/

59, 2270--2274 (2005)

Source: <http://www.insectscience.org/6.46>

International Journal of Basic and Applied Biology

Volume 3, Issue 1; January-March, 2016, pp. 33-35

In Vitro Studies to Evaluate the Antistaphylococcal Aureus Activities of Propolis: A Wonder Honey Bee Product in Comparison with Antibiotics: Ampicillin and Amoxycillin

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<sup>1</sup>Department of Zoology, Panjab University, Chandigarh-160014, INDIA

<sup>2</sup>Department of Biotechnology (UIET) Panjab University, Chandigarh-160014, INDIA

Abstract

**Aims:** Propolis: A wonder honey bee product is a natural resinous substance called Bee Glue, collected by bees from the exudates and secretions of plants. The aim of the present study was to determine the antistaphylococcal aureus activities of propolis.

**Methods and Results:** The antibacterial activity of propolis was determined by well diffusion and Macro dilution methods using ethanolic, methanolic and water extracts. Different extracts of Propolis at the concentrations of 15mg/ml, 30mg/ml, 45mg/ml, 60mg/ml and 75mg/ml were prepared and 50ul was added to the wells. After growth, zones of inhibition were determined and compared with antibiotic Ampicillin (10µg) and Amoxycillin (30µg). The ethanolic extract of propolis showed maximum inhibition (14.3mm) as compared to the methanolic (12.6mm) and water extract (10.7mm). The zone of inhibition of the positive control was found to be maximum (15.9mm) for Ampicillin and (17.8mm) for Amoxycillin.

**Conclusions:** Therefore from this study we can conclude that propolis can be used as a potential natural product for antibacterial therapeutics in the world of emerging drug resistance in microbes.

**Significance and Impact of the Study:** The antimicrobial properties warrant further studies on the clinical applications of propolis and some other honey bee products against microorganisms.

<https://academic.oup.com/gbe/advance-article/doi/10.1093/gbe/evz018/5318327>

Brock A Harpur, M Marta Guarna, Elizabeth Huxter, Heather Higo, Kyung-Mee Moon, Shelley E Hoover, Abdullah Ibrahim, Andony P Melathopoulos, Suresh Desai, Robert W Currie, Stephen F Pernal, Leonard J Foster, Amro Zayed;

Integrative Genomics Reveals the Genetics and Evolution of the Honey Bee's Social Immune System, *Genome Biology and Evolution*, , evz018,

<https://doi.org/10.1093/gbe/evz018>

Social organisms combat pathogens through individual innate immune responses or through social immunity—behaviors among individuals that limit pathogen transmission within groups. Although we have a relatively detailed understanding of the genetics and evolution of the innate immune system of animals, we know little about social immunity. Addressing this knowledge gap is crucial for understanding how life-history traits influence immunity, and identifying if trade-offs exist between innate and social immunity. Hygienic behavior in the Western honey bee, *Apis mellifera*, provides an excellent model for investigating the genetics and evolution of social immunity in animals. This heritable, colony-level behavior is performed by nurse bees when they detect and remove infected or dead brood from the colony. We sequenced 125 haploid genomes from two artificially selected highly hygienic populations and a baseline unselected population. Genomic contrasts allowed us to identify a minimum of 73 genes tentatively associated with hygienic behavior. Many genes were within previously discovered QTLs associated with hygienic behavior and were predictive of hygienic behavior within the unselected population. These genes were often involved in neuronal development and sensory perception in solitary insects. We found that genes associated with hygienic behavior have evidence of positive selection within honey bees (*Apis*), supporting the hypothesis that social immunity contributes to fitness. Our results indicate that genes influencing developmental neurobiology and behavior in solitary insects may have been co-opted to give rise to a novel and adaptive social immune phenotype in honey bees.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5595881/>

Propolis envelope in *Apis mellifera* colonies supports honey bees against the pathogen, *Paenibacillus larvae* Renata S. Borba& Marla Spivak *Sci Rep*. 2017; 7: 11429.

Published online 2017 Sep 12. doi: 10.1038/s41598-017-11689-w

Honey bees have immune defenses both as individuals and as a colony (e.g., individual and social immunity). One form of honey bee social immunity is the collection of antimicrobial plant resins and the deposition of the resins as a propolis envelope within the nest. In this study, we tested the effects of the propolis envelope as a natural defense against *Paenibacillus larvae*, the causative agent of American foulbrood (AFB) disease. Using colonies with and without a propolis envelope, we quantified: 1) the antimicrobial activity of larval food fed to 1-2 day old larvae; and 2) clinical signs of AFB. Our results show that the antimicrobial activity of larval food was significantly higher when challenged colonies had a propolis envelope compared to colonies without the envelope. In addition, colonies with a propolis envelope had significantly reduced levels of AFB clinical signs two months following challenge. Our results indicate that the propolis envelope serves as an antimicrobial layer around the colony that helps protect the brood from bacterial pathogen infection, resulting in a lower colony-level infection load.

The Science of Nature April 2017, 104:29

Firewalls in bee nests-survival value of propolis walls of wild Cape honeybee (*Apis mellifera capensis*)

Geoff Tribe Jürgen Tautz Karin Sternberg Jenny Cullinan

First Online: 13 March 2017 Received: 17 February 2017 Accepted: 19 February 2017 DOI:

<https://link.springer.com/article/10.1007%2Fs00114-017-1449-5>

The Cape bee is endemic to the winter rainfall region of South Africa where fires are an integral part of the ecology of the fynbos (heathland) vegetation. Of the 37 wild nests in pristine Peninsula Sandstone Fynbos in the Cape Point section of Table Mountain National Park that have been analyzed so far, only 22 could be accessed sufficiently to determine the existence of a propolis wall of which 68% had propolis walls which entirely enclosed their openings. The analysis of the 37 wild nests revealed that 78% occurred under boulders or in clefts within rocks, 11% in the ground, 8% in tree cavities, and 3% within shrubs. The analysis of 17 of these nests following a fire within the park revealed that the propolis walls materially protected the nests and retarded the fire with all the colonies surviving. The bees responded to the smoke by imbibing honey and retreating to the furthest recess of their nest cavity. The bees were required to utilize

this honey for about 3 weeks after which fire-loving plants appeared and began flowering. Considerable resources were utilized in the construction of the propolis walls, which ranged in thickness from 1.5 to 40 mm (mean 5 mm). Its physical environment determines the nesting behavior of the Cape bee. The prolific use of propolis serves to insulate the nest from extremes of temperature and humidity, restricts entry, camouflages the nest, and acts as an effective fire barrier protecting nests established mostly under rocks in vegetation subjected to periodic fires.

<http://www.mdpi.com/2075-4450/8/1/15/htm>

Insects 2017, 8(1), 15; doi:10.3390/insects8010015

Inside Honeybee Hives: Impact of Natural Propolis on the Ectoparasitic Mite *Varroa destructor* and Viruses  
Nora Drescher 1, Alexandra-Maria Klein 2, Peter Neumann 3,4, Orlando Yañez 3,4 and Sara D. Leonhardt  
Received: 14 July 2016 / Accepted: 22 January 2017 / Published: 6 February 2017

Social immunity is a key factor for honeybee health, including behavioral defense strategies such as the collective use of antimicrobial plant resins (propolis). While laboratory data repeatedly show significant propolis effects, field data are scarce, especially at the colony level. Here, we investigated whether propolis, as naturally deposited in the nests, can protect honeybees against ectoparasitic mites *Varroa destructor* and associated viruses, which are currently considered the most serious biological threat to European honeybee subspecies, *Apis mellifera*, globally. Propolis intake of 10 field colonies was manipulated by either reducing or adding freshly collected propolis. Mite infestations, titers of deformed wing virus (DWV) and sacbrood virus (SBV), resin intake, as well as colony strength were recorded monthly from July to September 2013. We additionally examined the effect of raw propolis volatiles on mite survival in laboratory assays. Our results showed no significant effects of adding or removing propolis on mite survival and infestation levels. However, in relation to *V. destructor*, DWV titers increased significantly less in colonies with added propolis than in propolis-removed colonies, whereas SBV titers were similar. Colonies with added propolis were also significantly stronger than propolis-removed colonies. These findings indicate that propolis may interfere with the dynamics of *V. destructor*-transmitted viruses, thereby further emphasizing the importance of propolis for honeybee health.

<https://academic.oup.com/jee/advance-article/doi/10.1093/jee/toy363/5199372>

Textured Hive Interiors Increase Honey Bee (Hymenoptera: Apidae) Propolis-Hoarding Behavior  
Cynthia R L Hodges Keith S Delaplane Berry J Brosi Journal of Economic Entomology, 28 November 2018  
Numerous papers have shown that propolis contributes favorably to worker honey bee (*Apis mellifera* L.) immune response and colony social immunity. Moreover, resin-foraging specialists are more sensitive than pollen foragers to tactile information in the nest interior, and they respond to these stimuli by collecting more resin. In this study, we show that in-hive propolis deposition is increased, compared with nonmodified controls, with any one of the three methods for increasing textural complexity of hive wall interior surfaces: 1) plastic propolis trap material stapled to wall interior, 2) parallel saw kerfs cut into wall interior, or 3) roughening wall interior with a mechanized wire brush. Pairwise comparisons showed that propolis deposition was not significantly different among the three textural treatments; however, textural treatments interacted with time to show a more consistent benefit from plastic propolis trap material or roughened interior surface over saw kerfs. Although direct health benefits were not measured, this work shows that it is comparatively simple to increase propolis deposition above background levels by increasing textural stimuli in hive interiors.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5595881/>

Propolis envelope in *Apis mellifera* colonies supports honey bees against the pathogen, *Paenibacillus larvae*  
Renata S. Borba & Marla Spivak Sci Rep. 2017; 7: 11429. Published online 2017 Sep 12.

<https://www.nature.com/articles/s41598-017-11689-w>

Honey bees have immune defenses both as individuals and as a colony (e.g., individual and social immunity). One form of honey bee social immunity is the collection of antimicrobial plant resins and the deposition of the resins as a propolis envelope within the nest. In this study, we tested the effects of the propolis envelope as a natural defense against *Paenibacillus larvae*, the causative agent of American foulbrood (AFB) disease. Using colonies with and without a propolis envelope, we quantified: 1) the antimicrobial activity of larval food fed to 1-2 day old larvae; and 2) clinical signs of AFB. Our results show that the antimicrobial activity of larval food was significantly higher when challenged colonies had a propolis

envelope compared to colonies without the envelope. In addition, colonies with a propolis envelope had significantly reduced levels of AFB clinical signs two months following challenge. Our results indicate that the propolis envelope serves as an antimicrobial layer around the colony that helps protect the brood from bacterial pathogen infection, resulting in a lower colony-level infection load.

## Hive magnetic materials and ambient electromagnetic radiation

**COMMENT: As bees are sensitive to the earth's magnetic field, there is a possibility that magnetic materials in the hive affect natural comb building.**

Radio-Frequency Electromagnetic Field Exposure of Western Honey Bees

Arno Thielens Mark K. Greco, Leen Verloock, Luc Martens & Wout Joseph

Scientific Reports volume 10, Article number: 461 (2020) <https://www.nature.com/articles/s41598-019-56948-0>

**ABSTRACT** Radio-frequency electromagnetic fields (RF-EMFs) can be absorbed in all living organisms, including Western Honey Bees (*Apis Mellifera*). This is an ecologically and economically important global insect species that is continuously exposed to environmental RF-EMFs. This exposure is studied numerically and experimentally in this manuscript. To this aim, numerical simulations using honey bee models, obtained using micro-CT scanning, were implemented to determine RF absorbed power as a function of frequency in the 0.6 to 120 GHz range. Five different models of honey bees were obtained and simulated: two workers, a drone, a larva, and a queen. The simulations were combined with in-situ measurements of environmental RF-EMF exposure near beehives in Belgium in order to estimate realistic exposure and absorbed power values for honey bees. Our analysis shows that a relatively small shift of 10% of environmental incident power density from frequencies below 3 GHz to higher frequencies will lead to a relative increase in absorbed power of a factor higher than 3.

Shepherd S, Hollands G, Godley VC, Sharkh SM, Jackson CW, Newland PL (2019)

Increased aggression and reduced aversive learning in honey bees exposed to extremely low frequency electromagnetic fields.

PLoS ONE 14(10): e0223614. <https://doi.org/10.1371/journal.pone.0223614>

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0223614>

**ABSTRACT** Honey bees, *Apis mellifera*, are a globally significant pollinator species and are currently in decline, with losses attributed to an array of interacting environmental stressors. Extremely low frequency electromagnetic fields (ELF EMFs) are a lesser-known abiotic environmental factor that are emitted from a variety of anthropogenic sources, including power lines, and have recently been shown to have a significant impact on the cognitive abilities and behaviour of honey bees. Here we have investigated the effects of field-realistic levels of ELF EMFs on aversive learning and aggression levels, which are critical factors for bees to maintain colony strength. Bees were exposed for 17 h to 100  $\mu$ T or 1000  $\mu$ T ELF EMFs, or a sham control. A sting extension response (SER) assay was conducted to determine the effects of ELF EMFs on aversive learning, while an intruder assay was conducted to determine the effects of ELF EMFs on aggression levels. Exposure to both 100  $\mu$ T and 1000  $\mu$ T ELF EMF reduced aversive learning performance by over 20%. Exposure to 100  $\mu$ T ELF EMFs also increased aggression scores by 60%, in response to intruder bees from foreign hives. These results indicate that short-term exposure to ELF EMFs, at levels that could be encountered in bee hives placed under power lines, reduced aversive learning and increased aggression levels. These behavioural changes could have wider ecological implications in terms of the ability of bees to interact with, and respond appropriately to, threats and negative environmental stimuli.

Orientation of comb building by honeybees

Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology

Volume 147, Number 4 / December, 1982 Pages 495-501

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Accepted: 29 March 1982

## Summary

Upon entering a new home site a honeybee swarm is faced with the task of organizing the building activities of thousands of component bees so that several straight and parallel vertically oriented combs can be quickly and efficiently built. As a part of this organization process it is necessary for the bees to select and agree upon a planar orientation for the new combs.

This paper presents evidence that memory of a previously used comb direction influences the building of the new set of combs. Swarms which have recently moved into bait-hives (empty boxes placed in trees to attract feral swarms) tend to maintain the previously used comb direction when removed and forced to build new combs, whereas swarms which have occupied the bait-hives for a longer period (over 9 days) do not. Recent swarms predictably alter their comb building direction within the influence of an applied earthstrength magnetic field, indicating that honey bees are able to use the earth's magnetic field as a reference at the commencement of comb construction in a new hive.

## Mobile phone-induced honeybee worker piping

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### Abstract—

The worldwide maintenance of the honeybee has major ecological, economic, and political implications. In the present study, electromagnetic waves originating from mobile phones were tested for potential effects on honeybee behavior. Mobile phone handsets were placed in the close vicinity of honeybees. The sound made by the bees was recorded and analyzed. The audiograms and spectrograms revealed that active mobile phone handsets have a dramatic impact on the behavior of the bees, namely by inducing the worker piping signal. In natural conditions, worker piping either announces the swarming process of the bee colony or is a signal of a disturbed bee colony.

*Apidologie* (2011) 42:270–279 Original article

*J. exp. Biol.* 126, 375-387 (1986) 375

## IRON-CONTAINING CELLS IN THE HONEY-BEE (*APIS MELLIFERA*) I. ADULT MORPHOLOGY AND PHYSIOLOGY

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### SUMMARY

Particulate iron was found within the trophocytes of the fat body of the adult honey-bee. These iron granules differed in their structure and composition from iron granules found in other biological systems. The granules had an average diameter of  $0.32 \pm 0.07$  microns and were composed of iron, calcium and phosphorus in a noncrystalline arrangement. The granules were apparently randomly distributed within the cytoplasm of the cells, and were not associated with any particular cellular organelle. Electron microscopy revealed the presence of cell junctions between the trophocytes. In tissues treated with colloidal lanthanum, 20-nm gaps were seen between the outer leaflets of the cells forming the cell junction. Physiological studies showed that these cells are electrically coupled, but the coupling ratio is low, as a result of extensive coupling to many cells.

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How do honeybees use their magnetic compass? Can they see the North?

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### Abstract

While seeking food sources and routes back to their hive, bees make use of their advanced nervous and sensory capacities, which underlie a diverse behavioral repertoire. One of several honeybee senses that is both exceptional and intriguing is magnetoreception – the ability to perceive the omnipresent magnetic field



(MF) of the Earth. The mechanism by which animals sense MFs has remained fascinating as well as elusive because of the intricacies involved, which makes it one of the grand challenges for neural and sensory biology. However, investigations in recent years have brought substantial progress to our understanding of how such magneto-receptor(s) may work. Some terrestrial animals (birds) are reported to be equipped even with a dual perception system: one based on diminutive magnetic particles – in line with the original model which has also always been hypothesized for bees – and the other one, as the more recent model describes, based on a sensitivity of some photochemical reactions to MF (radical-pair or chemical mechanism). The latter model postulates a close link to vision and supposes that the animals can see the position of the geomagnetic North as a visible pattern superimposed on the picture of the environment. In recent years, a growing body of evidence has shown that radical-pair magnetoreception might also be used by insects. It is realistic to expect that such evidence will inspire a re-examination and extension or confirmation of established views on the honeybee magnetic-compass mechanism. However, the problem of bee magnetoreception will not be solved at the moment that a receptor is discovered. On the contrary, the meaning of magnetoreception in insect life and its involvement in the orchestration of other senses is yet to be fully understood. The crucial question to be addressed in the near future is whether the compass abilities of the honeybee could suffer from radio frequency (RF) smog accompanying modern civilization and whether the fitness of this dominant pollinator might be affected by RF fields. The goal of this review is to provide an overview of the path that the behavioral research on honeybee magnetoreception has taken and to discuss it in the context of contemporary data obtained on other insects.

Does the Earth's Magnetic Field Serve as a Reference for Alignment of the Honeybee Waggle Dance?

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Abstract

The honeybee (*Apis mellifera*) waggle dance, which is performed inside the hive by forager bees, informs hive mates about a potent food source, and recruits them to its location. It consists of a repeated figure-8 pattern: two oppositely directed turns interspersed by a short straight segment, the “waggle run”. The waggle run consists of a single stride emphasized by lateral wagging motions of the abdomen. Directional information pointing to a food source relative to the sun's azimuth is encoded in the angle between the waggle run line and a reference line, which is generally thought to be established by gravity. Yet, there is tantalizing evidence that the local (ambient) geomagnetic field (LGMF) could play a role. We tested the effect of the LGMF on the recruitment success of forager bees by placing observation hives inside large Helmholtz coils, and then either reducing the LGMF to 2% or shifting its apparent declination. Neither of these treatments reduced the number of nest mates that waggle dancing forager bees recruited to a feeding station located 200 m north of the hive. These results indicate that the LGMF does not act as the reference for the alignment of waggle-dancing bees.

Lambinet V, Hayden ME, Bieri M, Gries G (2014) Does the Earth's Magnetic Field Serve as a Reference for Alignment of the Honeybee Waggle Dance? PLoS ONE 9(12): e115665.

doi:10.1371/journal.pone.0115665

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Effect of electromagnetic radiations on brooding, honey production and foraging behavior of European honeybees (*Apis mellifera* L.)

Pramod Mall & Yogesh Kumar

Abstract

The present investigation was carried out at HREC, Dhakrani, Dehradun during August-December, 2010. For the experiment three treatments, i) Colonies below the tower, ii) Colonies equipped with cell phone and iii) Colonies without cell phone were taken into consideration. To quantify the effect of electromagnetic radiation (EMR), all the treatments having different radiation level were maintained. It was observed that maximum brood area was found in control colonies (560.36 cm<sup>2</sup>) followed by the colonies kept near the tower 537.85 cm<sup>2</sup> and lowest brood area (534.81 cm<sup>2</sup>) was observed in the colonies equipped with cell phone. The average honey production was found to be highest (14.43 kg/hives) in the colonies placed near the tower followed by cell phone equipped colonies (13.76 kg/hive), while control colonies produced 12.80 kg/hive honey in first harvesting. There was no remarkable change in the nectar and pollen gathering

behaviour of foragers and sufficient pollen and nectar stored in the colonies during the course of study. Therefore, in the light of above findings conclusion can be drawn that there is no apparent effect of EMR on brooding, honey production and foraging behaviour of *Apis mellifera* colonies.

Effect of electromagnetic radiation of cell phone tower on foraging behaviour of Asiatic honey bee, *Apis cerana* F. (Hymenoptera: Apidae)

Ritu Ranjan Taye, Mukul Kumar Deka, Aatur Rahman and Manha Bathari

Journal of Entomology and Zoology Studies 2017; 5(3): 1527-1529

<http://www.entomoljournal.com/archives/2017/vol5issue3/PartU/5-3-142-590.pdf>

The effect of electromagnetic radiation (EMR) of cell phone tower on foraging behaviour of honey bee, *Apis cerana* F. was studied at Assam Agricultural University, Jorhat from December to May during 2012-13 and 2013-14 at an interval of 15 days. To quantify the effect of EMR, five treatments were placed at varying distance from the tower having different radiation level. The present study results revealed that the peak foraging behaviour of worker bees continues from March to May. The foraging behaviour of worker bees were observed maximum in colonies placed at 500m followed by 1000m, 300m and 200m and least at 100m distance from the tower. The results from the present investigation revealed that the *Apis cerana* colonies in close proximity to cell phones towers were most affected by the electromagnetic radiation emitted by the cell phone tower.

Extremely Low Frequency Electromagnetic Fields impair the Cognitive and Motor Abilities of Honey Bees

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Scientific Reports volume 8, Article number: 7932 (2018)

<https://www.nature.com/articles/s41598-018-26185-y>

Extremely low frequency electromagnetic field (ELF EMF) pollution from overhead powerlines is known to cause biological effects across many phyla, but these effects are poorly understood. Honey bees are important pollinators across the globe and due to their foraging flights are exposed to relatively high levels of ELF EMF in proximity to powerlines. Here we ask how acute exposure to 50 Hz ELF EMFs at levels ranging from 20–100  $\mu$ T, found at ground level below powerline conductors, to 1000–7000  $\mu$ T, found within 1 m of the conductors, affects honey bee olfactory learning, flight, foraging activity and feeding. ELF EMF exposure was found to reduce learning, alter flight dynamics, reduce the success of foraging flights towards food sources, and feeding. The results suggest that 50 Hz ELF EMFs emitted from powerlines may represent a prominent environmental stressor for honey bees, with the potential to impact on their cognitive and motor abilities, which could in turn reduce their ability to pollinate crops.

## Pheromone integrity and distribution

**COMMENT:** The honey bee nest is three-dimensionally structured as regards comb, its contents and the distribution of bees on it. It is plausible that the colony also has a three dimensional pheromonal structure that is important to its integrity and function. The position of the brood, the queen and the dance floor all have pheromonal associations. Johann Thür's concept of *Nestduftwärmebindung*, retention of nest *scent* and heat, may be relevant in this connection.

Alaux C., Maisonnasse A., Le Conte Y. (2010)

Pheromones in a superorganism: from gene to social regulation.

Vitamins & Hormones, 83: 401-423

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Abstract

Analogous to the importance of hormones in controlling organism homeostasis, pheromones play a major role in the regulation of group homeostasis at the social level. In social insects, pheromones coordinate the association of “unitary” organisms into a coherent social unit or so called “superorganism”. For many years, honeybees have been a convincing model for studying pheromone regulation of social life. In addition, with the recent sequencing of its genome, a global view of pheromone communication is starting to emerge, and

it is now possible to decipher this complex chemical language from the molecular to the social level. We review here the different pheromones regulating the main biological functions of the superorganism and detail their respective action on the genome, physiology and behavior of nestmates. Finally, we suggest some future research that may improve our understanding of the remarkably rich syntax of pheromone communication at the social level.

#### Behavioral Ecology and Sociobiology

October 2014, Volume 68, Issue 10, pp 1693-1700

Distance from the queen affects workers' selfish behaviour in the honeybee (*A. mellifera*) colony

Margarita Orlova, Abraham Hefetz

#### Abstract

It has been postulated that spatial organization of the colony, in addition to biological and social factors, influences colony life in honeybees. In this study, we examine the influence of workers' distance from the queen on their reproductive, pheromonal and behavioural characteristics. Our results demonstrate that with increasing distance from the queen, workers increasingly develop behavioural and reproductive traits characteristic of queenlessness, which are presumably the result of significant impairment in queen pheromone transmission. Having contemplated the alternative possibilities of inadequate transmission of queen signal and voluntary escape from queen control, we concluded, based on the behavioural and physiological data, that the former is far more probable than the latter. The dose-dependent manner of the queen pheromone action and its differential influence on various worker characteristics are discussed.

Veterinarni Medicina, 56, 2011 (6): 265-273

Chemical communication in the honeybee (*Apis mellifera*L.): a review

M. Trhlin, J. Rajchard

#### ABSTRACT:

An important area of physiology of the honeybee (*Apis mellifera*) is chemical communication between individuals and castes in the swarm, which maintains its integrity and function. The highly complex social organization of honeybees is mediated through pheromones. Releaser pheromones cause rapid changes in the behaviour of the recipient, while primer pheromones have relatively slow and long-term effects on the physiology and behaviour of the recipient. Queen retinue pheromone (QRP) is a blend of the nine compounds (9-oxo-(E)-2-decenoic acid, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid, methyl p-hydroxybenzoate, 4-hydroxy-3-methoxyphenylethanol, methyl oleate, coniferyl alcohol, palmityl alcohol, and linolenic acid) and acts as a releaser pheromone by attracting worker bees to the queen. QRP also acts as a primer pheromone by physiologically inhibiting the ovary development of worker bees. An essential component of QRP, 9-oxo-(E)-2-decenoic acid, acts as a long-distance sex pheromone. Defensive behaviour of honeybees is induced and modulated by alarm pheromones. The essential alarm pheromone component is isopentyl acetate (IPA). The unsaturated derivative of IPA, 3-methyl-2-buten-1-yl acetate, was found in colonies of Africanized honeybees. The Nasanov gland of worker bees produces a pheromone (a blend of nerol, geraniol, (E)- and (Z)-citral, nerolic acid, geranic acid and (E,E)-farnesol) that acts as an attracting signal. This pheromone is used for aggregation (during swarming). Adult worker bees also produce a substance, ethyl oleate, that has a priming effect. Ethyl oleate is produced by adult forager bees and acts as a chemical inhibitory factor to delay age at onset of foraging (the presence of older worker bees causes a delayed onset of foraging in younger individuals). Chemical cues on the surface of larvae called a brood pheromone (ethyl and methyl esters of palmitic, linoleic, linolenic, stearic, and oleic acids, E- $\beta$ -ocimene) are important in the communication between brood and worker bees. This pheromone modulates the feeding behaviour of worker bees, inhibits the activation of the worker ovary, induces worker bees to cap brood cells, increases the activity of the hypopharyngeal glands of nurse bees and modulates the behavioural maturation of worker bees.

Maisonnasse et al. Frontiers in Zoology 2010, 7:18

<http://www.frontiersinzoology.com/content/7/1/18>

#### Open Access RESEARCH

New insights into honey bee (*Apis mellifera*) pheromone communication. Is the queen mandibular pheromone alone in colony regulation?

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#### Abstract

**Background:** In social insects, the queen is essential to the functioning and homeostasis of the colony. This influence has been demonstrated to be mediated through pheromone communication. However, the only social insect for which any queen pheromone has been identified is the honey bee (*Apis mellifera*) with its well-known queen mandibular pheromone (QMP). Although pleiotropic effects on colony regulation are accredited to the QMP, this pheromone does not trigger the full behavioral and physiological response observed in the presence of the queen, suggesting the presence of additional compounds. We tested the hypothesis of a pheromone redundancy in honey bee queens by comparing the influence of queens with and without mandibular glands on worker behavior and physiology.

**Results:** Demandibulated queens had no detectable (E)-9-oxodec-2-enoic acid (9-ODA), the major compound in QMP, yet they controlled worker behavior (cell construction and queen retinue) and physiology (ovary inhibition) as efficiently as intact queens.

**Conclusions:** We demonstrated that the queen uses other pheromones as powerful as QMP to control the colony. It follows that queens appear to have multiple active compounds with similar functions in the colony (pheromone redundancy). Our findings support two hypotheses in the biology of social insects: (1) that multiple semiochemicals with synonymous meaning exist in the honey bee, (2) that this extensive semiochemical vocabulary exists because it confers an evolutionary advantage to the colony.

J Chem Ecol. 2005 Nov;31(11):2731-45. Epub 2005 Oct 25.

Pheromone communication in the honeybee (*Apis mellifera* L.).

Slessor KN, Winston ML, Le Conte Y.

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#### Abstract

Recent studies have demonstrated a remarkable and unexpected complexity in social insect pheromone communication, particularly for honeybees (*Apis mellifera* L.). The intricate interactions characteristic of social insects demand a complex language, based on specialized chemical signals that provide a syntax that is deeper in complexity and richer in nuance than previously imagined. Here, we discuss this rapidly evolving field for honeybees, the only social insect for which any primer pheromones have been identified. Novel research has demonstrated the importance of complexity, synergy, context, and dose, mediated through spatial and temporal pheromone distribution, and has revealed an unprecedented wealth of identified semiochemicals and functions. These new results demand fresh terminology, and we propose adding "colony pheromone" and "passenger pheromone" to the current terms sociochemical, releaser, and primer pheromone to better encompass our growing understanding of chemical communication in social insects.

Brood Pheromone Modulation of Pollen Forager Turnaround Time in the Honey Bee (*Apis mellifera* L.)

Journal of Insect Behavior

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Pages 173-180

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Foraging for pollen is an important behavior of the honey bee because pollen is their sole source of protein. Through nurse bees, larvae are the principal consumers of pollen. Fatty acid esters extractable from the surface of larvae, called brood pheromone, release multiple colony-level and individual foraging behaviors increasing pollen intake. In this study pollen forager turnaround time was measured in observation hives supplemented with brood pheromone versus a blank control treatment. Treatment with brood pheromone significantly decreased pollen forager turnaround time in the hive between foraging bouts by approximately 72%. Concurrently, brood pheromone increased the ratio of pollen to non-pollen foragers entering colonies. Brood pheromone has been shown to release most of the mechanisms known to increase pollen intake by colonies acting as an important regulator of colony foraging decisions and growth.

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Queen and young larval pheromones impact nursing and reproductive physiology of honey bee (*Apis mellifera*) workers

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Abstract

Several insect pheromones are multifunctional and have both releaser and primer effects. In honey bees (*Apis mellifera*), the queen mandibular pheromone (QMP) and  $\epsilon$ -beta-ocimene ( $\epsilon\beta$ ), emitted by young worker larvae, have such dual effects. There is increasing evidence that these multifunctional pheromones profoundly shape honey bee colony dynamics by influencing cooperative brood care, a fundamental aspect of eusocial insect behavior. Both QMP and  $\epsilon\beta$  have been shown to affect worker physiology and behavior, but it has not yet been determined if these two key pheromones have interactive effects on hypopharyngeal gland (HPG) development, actively used in caring of larvae, and ovary activation, a component of worker reproductive physiology. Experimental results demonstrate that both QMP and  $\epsilon\beta$  significantly suppress ovary activation compared to controls but that the larval pheromone is more effective than QMP. The underlying reproductive anatomy (total ovarioles) of workers influenced HPG development and ovary activation, so that worker bees with more ovarioles were less responsive to suppression of ovary activation by QMP. These bees were more likely to develop their HPG and have activated ovaries in the presence of  $\epsilon\beta$ , providing additional links between nursing and reproductive physiology in support of the reproductive ground plan hypothesis.

Behav Ecol Sociobiol DOI 10.1007/s00265-014-1811-y

Hydrocarbons emitted by waggle-dancing honey bees stimulate colony foraging activity by causing experienced foragers to exploit known food sources

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Received 7 February 2011–Revised 15 June 2011–Accepted 7 July 2011

Abstract–

Two hypotheses concerning the function of the hydrocarbons emitted by waggle-dancing bees are investigated in this study. First, we test the hypothesis that the waggle-dance compounds stimulate foraging behavior. In support of this hypothesis, the number of bee departures from a hive increased as much as 46% and the number of forager visits to a feeder station increased as much as 106% when the dance compounds were introduced into the hive. Second, we test the hypothesis that the waggle-dance compounds stimulate foraging by causing experienced foragers to depart the hive to exploit known food sources. In support of this hypothesis, individually marked foragers visited an empty feeder station both more often and in greater numbers following exposure to the waggle-dance compounds than following exposure to a control substance. The results of these experiments suggest that the waggle-dance compounds function as a foraging semiochemical, operating in synergy with the waggle dance itself.

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\*INRA, DIB-AGIB and Springer Science+Business Media B.V., 2011

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Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*)

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Smedal, B. Brynem, M. Kreibich, C. D. & Amdam, G. V. Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). *J. Exp. Biol.* **212**, 3795–3801

Honeybee (*Apis mellifera*) society is characterized by a helper caste of essentially sterile female bees called workers. Workers show striking changes in lifespan that correlate with changes in colony demography.

When rearing sibling sisters (brood), workers survive for 3-6 weeks. When brood rearing declines, worker lifespan is 20 weeks or longer. Insects can survive unfavorable periods on endogenous stores of protein and lipid. The glyco-lipoprotein vitellogenin extends worker bee lifespan by functioning in free radical defense, immunity and behavioral control. Workers use vitellogenin in brood food synthesis, and the metabolic cost of brood rearing (nurse load) may consume vitellogenin stores and reduce worker longevity. Yet, in addition to consuming resources, brood secretes a primer pheromone that affects worker physiology and behavior. Odors and odor perception can influence invertebrate longevity but it is unknown whether brood pheromone modulates vitellogenin stores and survival. We address this question with a 2-factorial experiment where 12 colonies are exposed to combinations of absence vs presence of brood and brood pheromone. Over an age-course of 24 days, we monitor the amount of vitellogenin stored in workers' fat body (adipose tissue). Thereafter, we track colony survival for 200 days. We demonstrate that brood rearing reduces worker vitellogenin stores and colony long-term survival. Yet also, we establish that the effects can result solely from exposure to brood pheromone. These findings indicate that molecular systems of extreme lifespan regulation are integrated with the sensory system of honeybees to respond to variation in a primer pheromone secreted from larvae.

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Wax combs mediate nestmate recognition by guard honeybees

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Abstract

Research has shown that the wax combs are important in the acquisition of colony odour in the honeybee, *Apis mellifera*. However, many of these studies were conducted in the laboratory or under artificial conditions. We investigated the role of the wax combs in nestmate recognition in the natural context of bees at colony entrances. Wax combs constructed by each experimental colony were swapped between hives and the acceptance of nestmate and non-nestmate forager workers was recorded before and after the swap, and in relation to a control hive not involved in the swap. We conducted the experiment twice, in consecutive years with three different colonies each time. Colonies that exchanged combs became more accepting of each other's workers than of workers from the third colony. The overall acceptance of comb swap non-nestmates increased significantly from 3% before the swap to 23% after the swap in the first year, and from 8 to 47% in the second year. This effect wore off within 3 weeks. Chemical analyses showed that the cuticular profile of non-nestmates involved in the experimental comb swap became more similar to each other after the swap, and that acceptance by guards of bees from different hives was negatively correlated with chemical distance between the hydrocarbon profiles of the workers from different hives.

Behavioral Ecology 2009 20(5):1007-1014; doi:10.1093/beheco/arp090

Queen reproductive state modulates pheromone production and queen-worker interactions in honeybees

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Abstract

The mandibular glands of queen honeybees produce a pheromone that modulates many aspects of worker honeybee physiology and behavior and is critical for colony social organization. The exact chemical blend produced by the queen differs between virgin and mated, laying queens. Here, we investigate the role of mating and reproductive state on queen pheromone production and worker responses. Virgin queens,

naturally mated queens, and queens instrumentally inseminated with either semen or saline were collected 2 days after mating or insemination. Naturally mated queens had the most activated ovaries and the most distinct chemical profile in their mandibular glands. Instrumentally inseminated queens were intermediate between virgins and naturally mated queens for both ovary activation and chemical profiles. There were no significant differences between semen- and saline-inseminated queens. Workers were preferentially attracted to the mandibular gland extracts from queens with significantly more activated ovaries. These studies suggest that the queen pheromone blend is modulated by the reproductive status of the queens, and workers can detect these subtle differences and are more responsive to queens with higher reproductive potential. Furthermore, it appears as if insemination substance does not strongly affect physiological characteristics of honeybee queens 2 days after insemination, suggesting that the insemination process or volume is responsible for stimulating these early postmating changes in honeybee queens.

Website: <http://entomology.ncsu.edu/grozinger>

## **Pollen nutrition and diversity; forage quality and abundance**

Honey Bee (Hymenoptera: Apidea) Pollen Forage in a Highly Cultivated Agroecosystem: Limited Diet Diversity and Its Relationship to Virus Resistance

Ge Zhang, Ashley L St. Clair, Adam Dolezal, Amy L Toth, Matthew O'Neal

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**ABSTRACT** Intensified agriculture reduces natural and seminatural habitats and plant diversity, reducing forage available to honey bees (*Apis mellifera* L. [Hymenoptera: Apidea]). In agricultural landscapes of Iowa, United States, we studied the impact of extrinsic agricultural intensification on the availability of pollen for honey bees by placing colonies next to soybean fields surrounded by either a low or high level of cultivation. The abundance and diversity of pollen returned to a colony were estimated by placing pollen traps on bee colonies during the summer and fall of 2015 and 2016. We observed no difference in abundance and diversity of pollen collected by colonies in either landscape, but abundance varied over time with significantly less collected in September. We explored if the most commonly collected pollen from these landscapes had the capacity to support honey bee immune health by testing if diets consisting of these pollens improved bee resistance to a viral infection. Compared to bees denied pollen, a mixture of pollen from the two most common plant taxa (*Trifolium* spp. L. [Fabales: Fabaceae] and *Chamaecrista fasciculata* (Michx.) Greene [Fabales: Fabaceae]) significantly reduced honey bee mortality induced by viral infection. These data suggest that a community of a few common plants was favored by honey bees, and when available, could be valuable for reducing mortality from a viral infection. Our data suggest a late season shortage of pollen may be ameliorated by additions of fall flowering plants, like goldenrod (*Solidago* spp. L. [Asterales: Asteraceae]) and sunflower (*Helianthus*, *Heliopsis*, and *Silphium* spp. [Asterales: Asteraceae]), as options for enhancing pollen availability and quality for honey bees in agricultural landscapes.

Honeybee nutrition is linked to landscape composition

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Ecology and Evolution 2014; 4(21): 4195–4206

Abstract

Declines in insect pollinators in Europe have been linked to changes in land use. Pollinator nutrition is dependent on floral resources (i.e., nectar and pollen), which are linked to landscape composition. Here, we present a stratified analysis of the nutritional composition of beebread in managed honeybee hives with a view to examining potential sources of variation in its nutritional composition. Specifically, we tested the hypothesis that beebread composition correlates with local land use and therefore available floral resources. The results demonstrated that the starch, lipid, and moisture contents of beebread are all highly conserved across hives, whereas levels of protein and nonreducing sugar increased as the year progressed, reducing sugars, however, decreased during the first half of the year and then increased toward the end. Local land

use around hives was quantified using data from the Countryside Survey 2007 Land Cover Map. Bee-bread protein content was negatively correlated with increasing levels of arable and horticultural farmland surrounding hives and positively correlated with the cover of natural grasslands and broadleaf woodlands. Reducing sugar content was also positively correlated with the amount of broad-leaved woodland in a 3 Km<sup>2</sup> radius from the hives. Previous studies on a range of invertebrates, including honeybees, indicate that dietary protein intake may have a major impact on correlates of fitness, including longevity and immune function. The finding that beebread protein content correlates with land use suggests that landscape composition may impact on insect pollinator well-being and provides a link between landscape and the nutritional ecology of socially foraging insects in a way not previously considered.

Bee World 86(1): 3-10 (2005) | March 2005

Pollen nutrition and colony development in honey bees: part II

IRENE KELLER, PETER FLURI AND ANTON IMDORF

Abstract

Pollen is the honey bees' main source of several important nutrients. Consequently, an adequate pollen supply is essential to ensure the long-term survival of a colony and to maintain its productivity. Part 1 of this 2-part review focuses on the botanical composition of bee-collected pollen and its protein and mineral content. Further, we discuss the impact of pollen on honey bee physiology and assess the pollen requirements of individual workers and larvae.

Protein content in larval diet affects adult longevity and antioxidant gene expression in honey bee workers

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Entomologia Experimentalis et Applicata

Volume 151, Issue 1, pages 19–26, April 2014

Abstract

In honey bees, adult longevity is strongly dependent on the quantity of dietary protein ingested after emergence, but relatively little is known about the role played by the protein content of larval diet. In total, 15 colonies of *Apis mellifera ligustica* Spinola (Hymenoptera: Apidae) bees with sister queens were randomly allocated to one of three groups (five colonies per group), provided with pollen substitutes (PS) with protein concentrations of 15, 25, or 35%, designated as PS15, PS25, and PS35, respectively. In a field experiment, we measured the PS consumption and collected samples for analyzing body protein content and gene expression. On day 29, groups of 50 newly emerged workers from each colony were obtained and confined in cages for measuring survival and longevity. Results showed that bees consumed significantly more PS15 and PS25 than PS35. However, the total protein intake of PS15 was less than that of the others. Increasing the protein available to larvae (PS35) significantly increased total accumulated protein before emergence, adult survival, and longevity. Furthermore, bees fed PS25 or PS35 tended to have higher mRNA levels for genes encoding antioxidant enzymes, providing a potential physiological mechanism for observed survival differences among the three dietary treatments. We conclude that protein content in larval diet could positively influence worker longevity likely because of the increased related antioxidant gene expression.

In press. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications*. <http://dx.doi.org/10.1890/14-1011.1>

Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds

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Abstract



In intensive farmland habitats, pollination of wild flowers and crops may be threatened by the widespread decline of pollinators. The honey bee decline, in particular, appears to result from the combination of multiple stresses, including diseases, pathogens, and pesticides. The reduction of semi-natural habitats is also suspected to entail floral resource scarcity for bees. Yet, the seasonal dynamics and composition of the honey bee diet remains poorly documented to date. In this study we studied the seasonal contribution of mass-flowering crops (rapeseed and sunflower) versus other floral resources, as well as the influence of nutritional quality and landscape composition on pollen diet composition over five consecutive years. From April to October, the mass of pollen and nectar collected by honey bees followed a bimodal seasonal trend, marked by two months of low food supply period between the two oilseed crops mass-flowerings (May for rapeseed and July for sunflower). Bees collected nectar mainly from crops while pollen came from a wide diversity of herbaceous and woody plant species, from semi-natural habitats or weeds in crops. Weed species constituted the bulk of the honey bee diet between the mass flowering crop periods (up to 40%) and are therefore suspected to play a critical role at this time period. The pollen diet composition was related to the nutritional value of the collected pollen and by the local landscape composition.

Our study highlights (i) a food supply depletion period of both pollen and nectar resources during late spring, contemporaneously with the demographic peak of honey bee populations, (ii) a high botanical richness of pollen diet, mostly proceeding from trees and weeds, and (iii) a pollen diet composition influenced by the local landscape composition. Our results therefore support the Agri-Environmental Schemes intended to promote honey bees and beekeeping sustainability through the enhancement of flower availability in agricultural landscapes.

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Original article

Comparison of pollen spectra collected by four different  
subspecies of the honey bee *Apis mellifera*\*

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Abstract – Colonies belonging to 4 subspecies of *Apis mellifera*, namely *A. m. capensis*, *A. m. ligustica*, *A. m. carnica* and *A. m. mellifera* were placed, one colony per subspecies, at 5 sites with a high floral diversity in the Taunus region in Germany. A total amount of 4008.3 g of pollen loads were trapped during 3 seasons and 214 different pollen types were identified. The comparison of pollen spectra did not result in a separation of the subspecies. Date of sampling and sampling site, however, had a major effect on the composition of pollen samples. Furthermore, subspecies were not significantly different in the structure of dominance, evenness and diversity of pollen types. We conclude that the investigated subspecies of *Apis mellifera* follow a generalist pollen foraging strategy which may be mainly shaped by natural selection to meet the nutritional and social requirements of populous colonies.

Diet effects on honeybee immunocompetence

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Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010) Diet effects on honeybee immunocompetence  
*Biol. Lett.* Published online doi:10.1098/rsbl.2009.0986

ABSTRACT

The maintenance of the immune system can be costly, and a lack of dietary protein can increase the

susceptibility of organisms to disease. However, few studies have investigated the relationship between protein nutrition and immunity in insects. Here, we tested in honeybees (*Apis mellifera*) whether dietary protein quantity (monofloral pollen) and diet diversity (polyfloral pollen) can shape baseline immunocompetence (IC) by measuring parameters of individual immunity (haemocyte concentration, fat body content and phenoloxidase activity) and glucose oxidase (GOX) activity, which enables bees to sterilize colony and brood food, as a parameter of social immunity. Protein feeding modified both individual and social IC but increases in dietary protein quantity did not enhance IC. However, diet diversity increased IC levels. In particular, polyfloral diets induced higher GOX activity compared with monofloral diets, including protein-rich diets. These results suggest a link between protein nutrition and immunity in honeybees and underscore the critical role of resource availability on pollinator health.

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Sagilie, R.R., & C. Breece -

#### EFFECTS OF POLLEN QUALITY (DIVERSITY) ON HONEY BEE PHYSIOLOGY, IMMUNOCOMPETENCE AND COLONY GROWTH

Poor nutrition or nutritional stress is one among several potential factors attributed for colony collapse disorder. Improved bee nutrition is key in dealing with most of the stress factors affecting honey bee health. Honey bee colonies that are poorly nourished are more susceptible to the gut parasite *Nosema ceranae* when compared to colonies that receive adequate nutrition (Eischen and Graham 2008). Protein deficiencies might compromise immune responses of an organism such as encapsulation (Siva-Jothy *et al.* 2005). Large monocultures and destruction of pollinator habitat have resulted in restricted choice of pollen diet in honey bees. Very little is known about effects of single-source pollen consumption for extended periods on honey bees. In this study we investigate and compare the effects of single source pollen consumption versus a multi-source pollen on honey bee physiology, immunocompetence and colony growth.

A large flight cage partitioned in to eighteen segments was used for this experiment. There were two treatments a) Single-source pollen and b) Multiple-source pollen. Each week comb area occupied by egg, larvae, pupae, honey, pollen and empty space was measured in each colony with a metered grid (Pankiw 2004). Also, each week 20 paint marked bees that were 7 days old were collected from each of the experimental colonies for hypopharyngeal gland protein estimation. Phenoloxidase and prophenoloxidase enzyme activity levels that are indicators of immunocompetence in honey bees were measured following the method of Laughton & Siva-Jothy (2010).

Nurse bee hypopharyngeal gland protein content was significantly lower in single-source pollen treatments when compared to multi-source pollen treatments ( $P < 0.01$ ). Multi-source pollen treatments had significantly higher colony growth in week's 4 and 5 ( $P < 0.01$ ) when compared to single-source pollen treatments. Both phenoloxidase and prophenoloxidase enzyme activities were significantly higher in multi-source pollen treatments when compared to single-source pollen treatments ( $P < 0.001$  and  $P < 0.01$  respectively) (Figure).

Department of Horticulture, Oregon State University, Corvallis, OR-97331-7304.

Sagili RR and Breece C (2012) Effects of pollen quality (diversity) on honey bee physiology, immunocompetence and colony growth. American Bee Research Conference, Riverdale, MD.

Di Pasquale G, Alaux C, Le Conte Y, Odoux J-F, Pioz M, Vaissière BE, et al. (2016)

Variations in the Availability of Pollen Resources Affect Honey Bee Health.

PLoS ONE 11(9): e0162818. doi:10.1371/journal.pone.0162818

Intensive agricultural systems often expose honey bees (*Apis mellifera*L.) to large temporal variations in the availability (quantity, quality and diversity) of nutritional resources. Such nutritional irregularity is expected to affect honey bee health. We therefore tested under laboratory conditions the effect of such variation in pollen availability on honey bee health (survival and nursing physiology—hypopharyngeal gland development and vitellogenin expression). We fed honey bees with different diets composed of pollen pellets collected by honey bees in an agricultural landscape of western France. Slight drops (5–10%) in the availability of oilseed rape (*Brassica napus*L.) pollen resulted in significant reductions of all tested variables. Despite some variations in taxonomic diversity and nutritional quality, the pollen mixes harvested over the season had a similar positive influence on honey bee health, except for the one collected in late July that

induced poor survival and nursing physiology. This period coincided with the mass-flowering of maize (*Zea mays*L.), an anemophilous crop which produces poor-quality pollen. Therefore, changes in bee health were not connected to variations in pollen diversity but rather to variations in pollen depletion and quality, such as can be encountered in an intensive agricultural system of western France. Finally, even though pollen can be available during the mass-flowering of some crops (e.g. maize), it can fail to provide bees with diet adequate for their development.

Ecological Applications, 25(4), 2015, pp. 881–890, 2015

Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds

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Abstract. In intensive farmland habitats, pollination of wild flowers and crops may be threatened by the widespread decline of pollinators. The honey bee decline, in particular, appears to result from the combination of multiple stresses, including diseases, pathogens, and pesticides. The reduction of semi-natural habitats is also suspected to entail floral resource scarcity for bees. Yet, the seasonal dynamics and composition of the honey bee diet remains poorly documented to date. In this study, we studied the seasonal contribution of massflowering crops (rapeseed and sunflower) vs. other floral resources, as well as the influence of nutritional quality and landscape composition on pollen diet composition over five consecutive years. From April to October, the mass of pollen and nectar collected by honey bees followed a bimodal seasonal trend, marked by a two-month period of low food supply between the two oilseed crop mass-flowerings (ending in May for rapeseed and July for sunflower). Bees collected nectar mainly from crops while pollen came from a wide diversity of herbaceous and woody plant species in semi-natural habitats or from weeds in crops. Weed species constituted the bulk of the honey bee diet between the mass flowering crop periods (up to 40%) and are therefore suspected to play a critical role at this time period. The pollen diet composition was related to the nutritional value of the collected pollen and by the local landscape composition. Our study highlights (1) a food supply depletion period of both pollen and nectar resources during late spring, contemporaneously with the demographic peak of honey bee populations, (2) a high botanical richness of pollen diet, mostly proceeding from trees and weeds, and (3) a pollen diet composition influenced by the local landscape composition. Our results therefore support the Agri-Environmental Schemes intended to promote honey bees and beekeeping sustainability through the enhancement of flower availability in agricultural landscapes.

Land use in the Northern Great Plains region of the U.S. influences the survival and productivity of honey bee colonies

Agriculture, Ecosystems and Environment 230 (2016) 139–149, 2016

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A B S T R A C T

The Northern Great Plains region of the US annually hosts a large portion of commercially managed U.S. honey bee colonies each summer. Changing land use patterns over the last several decades have contributed to declines in the availability of bee forage across the region, and the future sustainability of the region to support honey bee colonies is unclear. We examined the influence of varying land use on the survivorship

and productivity of honey bee colonies located in six apiaries within the Northern Great Plains state of North Dakota, an area of intensive agriculture and high density of beekeeping operations. Land use surrounding the apiaries was quantified over three years, 2010–2012, and survival and productivity of honey bee colonies were determined in response to the amount of bee forage land within a 3.2-km radius of each apiary. The area of uncultivated forage land (including pasture, USDA conservation program fields, fallow land, flowering woody plants, grassland, hay land, and roadside ditches) exerted a positive impact on annual apiary survival and honey production. Taxonomic diversity of bee-collected pollen and pesticide residues contained therein varied seasonally among apiaries, but overall were not correlated to large-scale land use patterns or survival and honey production. The predominant flowering plants utilized by honey bee colonies for pollen were volunteer species present in unmanaged (for honey bees), and often ephemeral, lands; thus placing honey bee colonies in a precarious situation for acquiring forage and nutrients over the entire growing season. We discuss the implications for land management, conservation, and beekeeper site selection in the Northern Great Plains to adequately support honey bee colonies and insure long term security for pollinator-dependent crops across the entire country.

Honey bee success predicted by landscape composition in Ohio, USA

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ABSTRACT

Foraging honey bees (*Apis mellifera* L.) can routinely travel as far as several kilometers from their hive in the process of collecting nectar and pollen from floral patches within the surrounding landscape. Since the availability of floral resources at the landscape scale is a function of landscape composition, apiculturists have long recognized that landscape composition is a critical determinant of honey bee colony success. Nevertheless, very few studies present quantitative data relating colony success metrics to local landscape composition. We employed a beekeeper survey in conjunction with GIS-based landscape analysis to model colony success as a function of landscape composition in the State of Ohio, USA, a region characterized by intensive cropland, urban development, deciduous forest, and grassland. We found that colony food accumulation and wax production were positively related to cropland and negatively related to forest and grassland, a pattern that may be driven by the abundance of dandelion and clovers in agricultural areas compared to forest or mature grassland. Colony food accumulation was also negatively correlated with urban land cover in sites dominated by urban and agricultural land use, which does not support the popular opinion that the urban environment is more favorable to honey bees than cropland.

Sponsler and Johnson (2015), Honey bee success predicted by landscape composition in Ohio, USA. *PeerJ* 3:e838; DOI 10.7717/peerj.838

<https://besjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/1365-2664.13447>

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Organic farming positively affects honeybee colonies in a flower-poor period in agricultural landscapes  
Dimitry Wintermantel

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Conventional farming has been implicated in global biodiversity and pollinator declines and organic farming is often regarded as a more ecological alternative. However, the effects of organic farming on honeybees remain elusive, despite honeybees' importance as pollinators of crops and wild plants.

Using 6 years of data from a large-scale study with fortnightly measurements of honeybee colony performance traits (10 apiaries per year distributed across a 435 km<sup>2</sup> large research site in France), we related worker brood area, number of adult bees and honey reserves to the proportions of organic farmland in the surroundings of the hives at two spatial scales (300 m and 1,500 m).

We found evidence that, at the local scale, organic farming increased both worker brood production and number of adult bees in the period of flower scarcity between the blooms of oilseed rape and sunflower (hereafter 'dearth period'). At the landscape scale, organic farming increased honey reserves during the dearth period and at the beginning of the sunflower bloom.

The results suggest that worker brood development benefitted from organic farming mostly through a more diverse diet due to an increase in the availability of diverse pollen sources in close proximity of their

hives. Reduced pesticide drift may have additionally improved bee survival. Honey reserves were possibly mostly affected by increased availability of melliferous flowers in foraging distance.

Synthesis and applications. Organic farming increases honeybee colony performance in a period of resource scarcity, likely through a continuous supply of floral resources including weeds, cover crops and semi-natural elements. We demonstrate how worker brood area increases in the critical dearth period (between the blooms of oilseed rape and sunflower). This has previously been linked to winter colony survival, suggesting that organic farmland may mitigate repercussions of intensive farming on colony vitality. We conclude that organic farming benefits a crucial crop pollinator with potential positive implications for agriculture in the wider landscape.

## Regulation of temperature and humidity

Comparison of colony performances of honeybee (*Apis Mellifera* L.) housed in hives made of different materials

Yasar Erdogan

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**ABSTRACT** Numerous flowering plants rely on bees for pollination. Any natural or anthropogenic factor that threatens colony survival can have a large impact on plant production. This study investigates the effects of manmade hives built from different materials and how these may influence colony growth. Ten each of three hive types were selected, wooden, polystyrene and composite insulated hives. Factors of adult bee numbers, brood development, nectar flow period weight gain, bee flight activity, aggression response, and honey yield were selected markers of hive development. Statistically, each hive type produced significant variation ( $p < .05$ ). The greatest overall productivity across all factors was for insulated hives made of composite material, with honey production approximately 35% times that of wood, 14% times that of polystyrene and in terms of the development of honeybee colonies, the average of other hives is 10.2 times.

The highly insulated hive of 'composite materials' was most productive but it does not say what the construction is, so this paper may be of dubious value.

5th COLOSS Conference – Montpellier, France, 14-15th September 2009

[COMMENT: see also J. Apicultural. Res. paper below]

Role of suboptimal brood rearing temperature in colony losses

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Abstract

The brood rearing temperature is one of the most precisely controlled physiological parameters in a honey bee colony. Due to its importance for colony health, normally only slight deviations from the optimal level may occur. Nevertheless, in particular situations the brood may be subject to conditions of suboptimal temperature, e.g. when the insufficient bee number cannot assure adequate thermoregulation. Moreover, in previous studies, adult bees reared at suboptimal temperature during pupal development, showed decrease in short-term learning and memory capacities. These bees could have difficulties to perform thermoregulation behaviour causing, again, reduced new brood temperature. To investigate the effects of the brood rearing temperature, a laboratory study was carried out. The larvae were reared in vitro at two different temperatures: 35°C (optimal) and 33°C (suboptimal) from the first hours of life until 15 days of age. Larval mortality, adult emergence and adult longevity were measured. Furthermore the susceptibility to the intoxication by Dymethoate was studied both on the larvae and on the adults emerged from the brood reared at the tested temperatures. The reduction of brood rearing temperature by 2°C had no effects either on larval

mortality, or on adult emergence rate, while adult longevity was strongly affected. Moreover adult bees, emerged from brood reared at the suboptimal temperature, responded quicker to intoxication by Dymethoate. Surprisingly, at the lower temperature, the larval LD50 (48h) was much higher and the response to the intoxication was delayed. In fact, after 24 hours, the mortality rate was quite stable at 35°C, while it was still rising at 33°C. This may be explained by the slower larval metabolism at the lower temperature, with the consequent slower active ingredient absorption. With this study we can conclude that adult honey bees deriving from brood reared at suboptimal temperature have lower fitness and are more susceptible to pesticide intoxication. We hypothesise that a slight poisoning in early spring i.e. in the conditions of low external temperatures, having apparently no important negative effects on the colony, can lead to suboptimal brood temperature, due to insufficient number of bees, and could affect the fitness of the adults that will emerge. Consequently, the next brood, reared by these adults, may be neglected. Therefore, the low-temperature-brood-rearing should be considered a significant stress factor and may play important role in bee/colony losses, even if its effects on the entire colony are delayed in time and difficult to link directly to the primary cause (slight bee loss in spring).

Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides

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Summary

Adult honey bees (*Apis mellifera*) usually maintain colony brood rearing temperature between 34-35°C by thermoregulation. The brood may, however, also be subjected to suboptimal temperature. Here we investigated whether a decrease of brood rearing temperature may have effects on larval mortality, adult emergence, longevity, morphology and susceptibility to poisoning by pesticides (dimethoate). Using the *in vitro* rearing protocol of Aupinel (2005), we were able for the first time to control the brood temperature not only during the pupal stage but also during the larval stage. Honey bee larvae were reared *in vitro* at 35°C (optimal) and 33°C (suboptimal) from 12 h after hatching for 15 days. Dimethoate was tested by ingestion either on 4-day old larvae or on 7-day old adults. Our results showed that lower rearing temperature had no significant effects on larval mortality and adult emergence, but adult bee mortality was strongly affected. Moreover, adult workers emerging at 33°C were significantly more susceptible to dimethoate. Larval LD50 (48 h) was, however, 28 times higher at 33°C than at 35°C. The striking differences between larvae and adults may be explained by differential larval metabolism at 33°C and resulting slower active ingredient absorption. We conclude that adult honey bees reared at even slightly suboptimal brood temperature may be more susceptible to pesticide poisoning and be characterised by reduced longevity. Thus, low temperature brood rearing could be another stress factor for colonies.

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Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development

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Tautz, J. Maier, S., Groh, C., Rössler, W. & Brockmann, A. (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *PNAS* 100(12) 7343-7347.

#### Abstract

To investigate the possible consequences of brood-temperature regulation in honey bee colonies on the quality of behavioral performance of adults, we placed honey bee pupae in incubators and allowed them to develop at temperatures held constant at 32°C, 34.5°C, and 36°C. This temperature range occurs naturally within hives. On emergence, the young adult bees were marked and introduced into foster colonies housed in normal and observation hives and allowed to live out their lives. No obvious difference in within-hive behavior was noted between the temperature-treated bees and the foster-colony bees. However, when the temperature-treated bees became foragers and were trained to visit a feeder 200 m from the hive, they exhibited clear differences in dance performance that could be correlated with the temperatures at which they had been raised: bees raised at 32°C completed only  $\approx 20\%$  of the dance circuits when compared with bees of the higher-temperature group. Also, the variance in the duration of the waggle phase is larger in 32°C-raised bees compared with 36°C-raised bees. All other parameters compared across all groups were not significantly different. One-trial learning and memory consolidation in the bees raised at different temperatures was investigated 1 and 10 min after conditioning the proboscis-extension reflex. Bees raised at 36°C performed as expected for bees typically classified as “good learners,” whereas bees raised at 32°C and 34.5°C performed significantly less well. We propose that the temperature at which pupae are raised will influence their behavioral performance as adults and may determine the tasks they carry out best inside and outside the hive.

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#### Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development

Claudia Groh, Jürgen Tautz, and Wolfgang Rössler\*

4268–4273  $\uparrow$  PNAS  $\uparrow$  March 23, 2004  $\uparrow$  vol. 101  $\uparrow$  no. 12

Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

Communicated by Bert Hölldobler, University of Würzburg, Würzburg, Germany, February 3, 2004 (received for review December 15, 2003)

Groh, C., Tautz, J. & Rössler, W. (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *PNAS* **101**(12) 4268–4273.

#### Abstract

Recent studies have shown that the behavioral performance of adult honey bees is influenced by the temperature experienced during pupal development. Here we explore whether there are temperature-mediated effects on the brain. We raised pupae at different constant temperatures between 29 and 37°C and performed neuroanatomical analyses of the adult brains. Analyses focused on sensory-input regions in the mushroom bodies, brain areas associated with higher-order processing such as learning and memory. Distinct synaptic complexes [microglomeruli (MG)] within the mushroom body calyces were visualized by using fluorophore-conjugated phalloidin and an antibody to synapsin. The numbers of MG were different in bees that had been raised at different temperatures, and these differences persisted after the first week of adult life. In the olfactory-input region (lip), MG numbers were highest in bees raised at the temperature normally maintained in brood cells (34.5°C) and significantly decreased in bees raised at 1°C below and above this norm. Interestingly, in the neighboring visual-input region (collar), MG numbers were less affected by temperature. We conclude that thermoregulatory control of brood rearing can generate area- and modality-specific effects on synaptic neuropils in the adult brain. We propose that resulting differences in the synaptic circuitry may affect neuronal plasticity and may underlie temperature-mediated effects on multimodal communication and learning.

Jones, J., Helliwell, P., Beekman, M., Maleszka, R. J. & Oldroyd, B. P. (2005) The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *J Comp Physiol A* **191** 1121–1129.

## Abstract

Honey bee workers maintain the brood nest of their colony within a narrow temperature range of  $34.5 \pm 1.5$  degrees C, implying that there are significant fitness costs if brood is reared outside the normal range. However, the effects of abnormal incubation temperatures are subtle and not well documented. Here we show that short-term learning and memory abilities of adult workers are affected by the temperature they experienced during pupal development. In contrast, long-term learning and memory is not significantly affected by rearing temperature. Furthermore, we could detect no effects of incubation temperature on fluctuating asymmetry, as a measure of developmental stability, in workers, queens or drones. We conclude that the most important consequence of abnormal rearing temperatures are subtle neural deficiencies affecting short-term memory rather than physical abnormalities.

Naturwissenschaften (2006) 93: 397–401

DOI 10.1007/s00114-006-0117-y

SHORT COMMUNICATION

Hannelie Human . Sue W. Nicolson .

Vincent Dietemann

Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest?

Human, H., Nicolson, S. W. & Dietmann, V. (2006) Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest? *Naturwissenschaften* **93** 397–401.

Received: 21 October 2005 / Accepted: 23 March 2006 / Published online: 3 May 2006

## Abstract

Honeybees are highly efficient at regulating the biophysical parameters of their hive according to colony needs. Thermoregulation has been the most extensively studied aspect of nest homeostasis. In contrast, little is known about how humidity is regulated in beehives, if at all. Although high humidity is necessary for brood development, regulation of this parameter by honeybee workers has not yet been demonstrated. In the past, humidity was measured too crudely for a regulation mechanism to be identified. We reassess this issue, using miniaturised data loggers that allow humidity measurements in natural situations and at several places in the nest. We present evidence that workers influence humidity in the hive. However, there are constraints on potential regulation mechanisms because humidity optima may vary in different locations of the nest. Humidity could also depend on variable external factors, such as water availability, which further impair the regulation. Moreover, there are trade-offs with the regulation of temperature and respiratory gas exchanges that can disrupt the establishment of optimal humidity levels. As a result, we argue that workers can only adjust humidity within sub-optimal limits.

Journal of Insect Physiology

Volume 54, Issue 12, December 2008, Pages 1516–1521

Hygropreference and brood care in the honeybee (*Apis mellifera*)

Michael B. Ellis, , Sue W. Nicolson, Robin M. Crewe, Vincent Dietemann

## Abstract

Terrestrial organisms need to limit evaporation from their bodies in order to maintain a homeostatic water balance. Owing to a large surface to volume ratio, arthropods are particularly susceptible to desiccation and have evolved behavioural and physiological mechanisms to conserve water. In social insects, water balance is also affected by the interactions between nestmates and by the architecture of the nest. For honeybees, humidity is particularly important for the brood because it affects the hatching success of eggs and because, unlike ants, honeybees cannot relocate their brood to parts of the nest with more favourable humidity. To advance the understanding of the water economy in honeybee nests, we investigated whether workers exhibit a hygropreference when exposed to a gradient of 24–90% relative humidity (RH) and whether the expression of this preference and their behaviour is affected by the presence of brood. The results show that young honeybee workers in the absence of brood exhibit a weak hygropreference for approximately 75% RH. When brood is present the expression of this preference is further weakened, suggesting that workers tend to the brood by distributing evenly in the gradient. In addition, fanning behaviour is shown to be triggered by an increase in humidity above the preferred level but not by a decrease. Our results suggest that humidity in honeybee colonies is actively controlled by workers.



Brood pupation temperature affects the susceptibility of honeybees (*Apis mellifera*) to infestation by tracheal mites (*Acarapis woodi*)

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Department of Zoology, Trinity College Dublin, Dublin 2, Ireland

Received 12 March 2004 – Revised 1 July 2004 – Accepted 26 July 2004

Published online 16 March 2005

Abstract–

An assessment was made of tracheal mite susceptibility in honeybees pupated at a low temperature. Using a laboratory bioassay, an experiment was conducted to compare the performance of newly-emerged (callow) bees raised at 30 °C with those raised at the more normal brood temperature of 34 °C. The reduced temperature caused a delay of over 5 days in the emergence of the bees from the brood cells. The callow bees raised at 30°C had over twice the mite prevalence level. The fecundity of the mites in the tracheae was similar for both temperature conditions. Increased susceptibility to tracheal mites resulting from reduced brood temperature may help to explain the mortality, in the temperature-stressed late winter/early spring period, of colonies with a moderate mite infestation in autumn. Further work is required to identify the mechanism responsible for this increased susceptibility.

**COMMENT:** The following dissertation shows the precision of the thermal regulation structure within the brood nest. The findings are briefly summarised in Jürgen Tautz's book 'The Buzz About Bees'.

URL: <http://www.opus-bayern.de/uni-wuerzburg/volltexte/2005/1590/>

Thermoregulation in the brood region of honeybees (*Apis mellifera carnica*)

Bujok, Brigitte

pdf-Format: Dokument 1.pdf (2.677 KB)

Institut: Theodor-Boveri-Institut für Biowissenschaften

Fakultät: Fakultät für Biologie

DDC-Sachgruppe: Biowissenschaften, Biologie

Dokumentart: Dissertation

Supervisor: Tautz, Jürgen (Prof. Dr.)

Language: Deutsch

Oral examination: 02.11.2005

Honey bees (*Apis mellifera carnica*) precisely regulate brood temperature in a range between 33 to 35°C, as the development of the larvae, especially the capped brood stages are very sensitive to temperature changes. The heat used for thermoregulation in the brood area is not a by-product of work, but an active time and energy consuming task called brood heating behaviour: worker bees pull their warm thoraces towards the brood caps and remain in that position for about 10 minutes to transfer their body heat to the brood caps and the brood (Bujok et al. 2002). As expected, thoracic temperatures of bees correlated with frequency of abdominal breathing movements; bees with high thoracic temperatures (above 40°C) showed abdominal movements of up to slightly over 8Hz.

Honey bee workers use empty cells neighbouring the capped brood cells for brood heating (Kleinhenz et al. 2003). Therefore, bees preferred visiting cells neighboured by numerous brood cells. Both duration of cell visits and thoracic temperature of the bee correlated with the number of neighboured brood cells. Older bees (at least 48h) showed significantly higher thoracic temperatures than younger bees (up to 48h old) immediately before and after their cell visits.

Dead capped brood were heated over several days and showed equivalent temperatures to living brood warmed by worker bees. In other cases, the brood temperature was lower, but still in the optimal temperature range of 33.5 to 35°C (Groh et al. 2004). In the experiments, the dead brood was killed by cold then warmed up again to 35°C before putting it into the observation hive. Capped brood cells filled with wax dummies were not heated and immediately cleared out. Mechanical signals from the brood seemed not to be important for the brood heating behaviour of the worker bees; observations with laser-vibrometry showed no signals coming from the brood demanding thermoregulation.

The antennae of the bees seem to be very important for effective brood heating, since during brood heating behaviour the antennae point to the capped brood surface. Worker bees with shortened antennae showed inferior brood heating behaviour, while brood temperature correlated with number of missing antenna segments. Additionally, the effectiveness of brood heating seems to be linked to a underlying laterality of the antennas function, when parts of the right antenna were amputated, brood heating was worse than when parts of the left antenna were amputated. With the amputation of the antenna the behaviour of the bees changed: control bees remained very quiet in the capped brood area, while bees with amputated antenna with similarly warm thoraces did not show the typical brood heating behaviour anymore.

In some observations brood temperature dropped for some hours and increased again in a way that can only be explained by positive feedback of brood heating of the worker bees. Brood heating appears to be a collective action.

COMMENT: Hive design affects heat dissipation and thus the colony's energy expenditure from its hard won honey stores.

The Journal of Experimental Biology 206, 353-358

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doi:10.1242/jeb.00082

Endothermic heat production in honeybee winter clusters

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Abstract

In order to survive cold northern winters, honeybees crowd tightly together in a winter cluster. Present models of winter cluster thermoregulation consider the insulation by the tightly packed mantle bees as the decisive factor for survival at low temperatures, mostly ignoring the possibility of endothermic heat production. We provide here direct evidence of endothermic heat production by 'shivering' thermogenesis. The abundance of endothermic bees is highest in the core and decreases towards the surface. This shows that core bees play an active role in thermal control of winter clusters. We conclude that regulation of both the insulation by the mantle bees and endothermic heat production by the inner bees is necessary to achieve thermal stability in a winter cluster.

<http://eprints.whiterose.ac.uk/141143/3/rsoc23122018-manuscriptD.pdf>

Mitchell, D (Accepted: 2018)

Thermal efficiency extends distance and variety for honey bee foragers: Analysis of the energetics of nectar collection and desiccation by *Apis mellifera*.

Journal of the Royal Society Interface. ISSN 1742-5689

The desiccation of nectar to produce honey by honey bees (*Apis mellifera* L.) is an energy intensive process, as it involves a quasi-isothermal change in the concentration of sugars from typically 20% to 80% by vaporisation (honey ripening). This analysis creates mathematical models for: the collected nectar to honey ratio; energy recovery ratio; honey energy margin; and the break-even distance, which includes the factors of nectar concentration and the distance to the nectar from the nest; energetics of desiccation; and a new factor, thermal energy efficiency of nectar desiccation (TEE). These models show a significant proportion of delivered energy in the nectar must be used in desiccation and that there is a strong connection between TEE and nest lumped thermal conductance with colony behaviour. They show the connection between TEE and honey bee colony success, or failure, in the rate of return, in terms of distance or quality of foraging. Consequently TEE is a key parameter in honey bee population and foraging modelling. For bee-keeping it quantifies the summer benefits of a key hive design parameter, hive thermal conductance and gives a sound theoretical basis for improving honey yields, as seen in expanded polystyrene hives

## **Suppression of reproductive swarming and associated beekeeping practices may adversely affect bee health**

Review article

Implications of horizontal and vertical pathogen transmission for honey bee epidemiology  
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University Park, PA 16802, USA

Fries, I. & Camazine, S. (2001) Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie* **32** 199–214.

(Received 15 January 2001; accepted 8 March 2001)

Abstract – The degree to which a disease evolves to be virulent depends, in part, on whether the pathogen is transmitted horizontally or vertically. Eusocial insect colonies present a special case since the fitness of the pathogen depends not only on the ability to infect and spread between individuals within a colony, but also on the ability to spread to new individuals in other colonies. In honey bees, intercolony transmission of pathogens occurs horizontally (by drifting or robbing) and vertically (through swarming). Vertical transmission is likely the most important route of pathogen infection of new colonies. Theory predicts that this should generally select for benign host-parasite relationships. Indeed, most honey bee diseases exhibit low virulence. The only major exception is American foulbrood (AFB). In light of current ideas in evolutionary epidemiology, we discuss the implications of horizontal and vertical pathogen transmission for virulence of AFB and other honey bee diseases.

*Apidologie* 32 (2001) 199–214 199

© INRA/DIB-AGIB/EDP Sciences, 2001

**COMMENT: What criteria bees use for polygyny reduction during queen replacement (after reproductive swarming or after queen loss) is not known.**

*Insectes sociaux*. 46 (1999) 372 – 377

Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality

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Received 21 September 1998; revised 19 January 1999; accepted 9 March 1999.

Summary.

The requeening process was investigated under emergency conditions in honey bee colonies (*Apis mellifera* L.). The progression of queen cell construction was closely monitored after removal of the mother queen, and the newly emerged queens were measured for several physical traits to quantify their reproductive potential (= quality). The results suggest that workers regulate the queen rearing process by differentially constructing cells. Workers built different numbers of queens cells from different ages of brood and non-randomly destroyed over half (53 %) of the initiated cells before their emergence. For those queens whose cells were not torn down, the variation in reproductive quality was limited, varying only slightly among age groups for queen size. Several hypotheses are discussed which might explain the adaptive benefit of worker regulation during queen rearing.

**COMMENT: Artificial queen replacement usually involves grafting larvae destined to be workers. The following studies compared queens from younger and older grafted larvae.**

The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth

J. Rangel, J. J. Keller, D. R. Tarpay

*Insectes Sociaux* February 2013, Volume 60, Issue 1, pp 65-73

Abstract

Reproduction in species of eusocial insects is monopolized by one or a few individuals, while the remaining colony tasks are performed by the worker caste. This reproductive division of labor is exemplified by honey bees (*Apis mellifera* L.), in which a single, polyandrous queen is the sole colony member that lays fertilized

eggs. Previous work has revealed that the developmental fate of honey bee queens is highly plastic, with queens raised from younger worker larvae exhibiting higher measures in several aspects of reproductive potential compared to queens raised from older worker larvae. Here, we investigated the effects of queen reproductive potential (“quality”) on the growth and winter survival of newly established honey bee colonies. We did so by comparing the growth of colonies headed by “high-quality” queens (i.e., those raised from young worker larvae, which are more queen-like morphologically) to those headed by “low-quality” queens (i.e., those raised from older worker larvae, which are more worker-like morphologically). We confirmed that queens reared from young worker larvae were significantly larger in size than queens reared from old worker larvae. We also found a significant positive effect of queen grafting age on a colony’s production of worker comb, drone comb, and stored food (honey and pollen), although we did not find a statistically significant difference in the production of worker and drone brood, worker population, and colony weight. Our results provide evidence that in honey bees, queen developmental plasticity influences several important measures of colony fitness. Thus, the present study supports the idea that a honey bee colony can be viewed (at least in part) as the expanded phenotype of its queen, and thus selection acting predominantly at the colony level can be congruent with that at the individual level.

<http://link.springer.com/article/10.1007/s00040-012-0267-1>

ANIMAL BEHAVIOUR, 2000,59,97–101

Article No. anbe.1999.1311, available online at <http://www.idealibrary.com> on

The influence of queen age and quality during queen replacement in honeybee colonies

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Department of Biology, Bucknell University

(Received 14 May 1999; initial acceptance 29 July 1999;

final acceptance 27 September 1999; MS. number: A8303R)

Honeybee, *Apis mellifera*, colonies replace their queens by constructing many queen cells and then eliminating supernumerary queens until only one remains. The ages of the queens and the variation in their reproductive potential are important factors in the outcome of such events. Selection would favour colonies that requeen as quickly as possible to minimize the brood hiatus, therefore selecting for queens reared from older larvae. Conversely, reproductive potential (queen ‘quality’) is maximized by rearing queens from younger larvae. This potential trade-off was tested during two phases of queen replacement, namely queen rearing and polygyny reduction. Our results suggest that queen age is a significant element during both queen rearing and polygyny reduction, whereas queen quality, at least to the magnitude tested in this experiment, has little impact on the outcome of either process. The rate of queen replacement therefore appears to be an important factor in the honeybee life cycle, and further mechanisms of potential importance during this life history transition are discussed.

The following paper shows that Varroa burdens are lower in colonies in small hives that have a high swarming rate (yet have cavity volumes the same as the average for natural nests in trees) compared with in large hives with a low swarming rate.

How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming

J. Carter Loftus, Michael L. Smith, Thomas D. Seeley\*

Department of Neurobiology and Behavior, Cornell University, Ithaca, New York, United States of America  
Abstract

The ectoparasitic mite, *Varroa destructor*, and the viruses that it transmits, kill the colonies of European honey bees (*Apis mellifera*) kept by beekeepers unless the bees are treated with miticides. Nevertheless, there exist populations of wild colonies of European honey bees that are persisting without being treated with miticides. We hypothesized that the persistence of these wild colonies is due in part to their habits of nesting in small cavities and swarming frequently. We tested this hypothesis by establishing two groups of colonies living either in small hives (42 L) without swarm-control treatments or in large hives (up to 168 L) with swarm-control treatments. We followed the colonies for two years and compared the two groups with respect to swarming frequency, *Varroa* infestation rate, disease incidence, and colony survival. Colonies in small hives swarmed more often, had lower *Varroa* infestation rates, had less disease, and had higher

survival compared to colonies in large hives. These results indicate that the smaller nest cavities and more frequent swarming of wild colonies contribute to their persistence without mite treatments.

Loftus JC, Smith ML, Seeley TD (2016) How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming. PLoS ONE 11(3): e0150362. doi:10.1371/journal.pone.0150362

<https://www.ncbi.nlm.nih.gov/pubmed/28486589> It appears to be open access only in the USA. Otherwise there is sci-hub.bz (sci-hub.cc seems to be down) for which one need only enter the DOI. Are Dispersal Mechanisms Changing the Host-Parasite Relationship and Increasing the Virulence of *Varroa destructor* (Mesostigmata: Varroidae) in Managed Honey Bee (Hymenoptera: Apidae) Colonies? Gloria DeGrandi-Hoffman, Fabiana Ahumada, and Henry Graham This forum article is an invited paper Subject Editor: E. Alan Cameron Received 15 December 2016; Editorial decision 24 March 2017 Environ Entomol. 2017 May 9. doi: 10.1093/ee/nvx077. [Epub ahead of print] Abstract *Varroa destructor* Anderson and Trueman) are a serious pest of European honey bees (*Apis mellifera* L.), and difficult to control in managed colonies. In our 11-mo longitudinal study, we applied multiple miticide treatments, yet mite numbers remained high and colony losses exceeded 55%. High mortality from varroa in managed apiaries is a departure from the effects of the mite in feral colonies where bees and varroa can coexist. Differences in mite survival strategies and dispersal mechanisms may be contributing factors. In feral colonies, mites can disperse through swarming. In managed apiaries, where swarming is reduced, mites disperse on foragers robbing or drifting from infested hives. Using a honey bee-varroa population model, we show that yearly swarming curtails varroa population growth, enabling colony survival for >5 yr. Without swarming, colonies collapsed by the third year. To disperse, varroa must attach to foragers that then enter other hives. We hypothesize that stress from parasitism and virus infection combined with effects that viruses have on cognitive function may contribute to forager drift and mite and virus dispersal. We also hypothesize that drifting foragers with mites can measurably increase mite populations. Simulations initialized with field data indicate that low levels of drifting foragers with mites can create sharp increases in mite populations in the fall and heavily infested colonies in the spring. We suggest new research directions to investigate factors leading to mite dispersal on foragers, and mite management strategies with consideration of varroa as a migratory pest.

Loftus JC, Smith ML, Seeley TD (2016) How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming.

PLoS ONE 11(3): e0150362. doi:10.1371/journal.pone.0150362

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0150362>

The ectoparasitic mite, *Varroa destructor*, and the viruses that it transmits, kill the colonies of European honey bees (*Apis mellifera*) kept by beekeepers unless the bees are treated with miticides. Nevertheless, there exist populations of wild colonies of European honey bees that are persisting without being treated with miticides. We hypothesized that the persistence of these wild colonies is due in part to their habits of nesting in small cavities and swarming frequently. We tested this hypothesis by establishing two groups of colonies living either in small hives (42 L) without swarm-control treatments or in large hives (up to 168 L) with swarm-control treatments. We followed the colonies for two years and compared the two groups with respect to swarming frequency, *Varroa* infestation rate, disease incidence, and colony survival. Colonies in small hives swarmed more often, had lower *Varroa* infestation rates, had less disease, and had higher survival compared to colonies in large hives. These results indicate that the smaller nest cavities and more frequent swarming of wild colonies contribute to their persistence without mite treatments.

## **Foul brood incidence is higher in managed colonies compared with ferals**

New Zealand Journal of Zoology, 1994, Vol. 21: 285-287

0301-4223/2103-0285 \$2.50/o' © The Royal Society of New Zealand 1994

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Incidence of American foulbrood infections in feral honey bee colonies in New Zealand

R. M. GOODWIN

A. TEN HOUTEN

J. H. PERRY

Goodwin, R. M., Ten Houten, A. & Perry, J. H. (1994) Incidence of American foulbrood infections in feral honey bee colonies in New Zealand. *NZ J. Zool.* 21 285-287

Apicultural Research Unit

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Abstract

Samples of forager honey bees (*Apis mellifera* L.) taken from the entrances of 109 feral colonies in New Zealand were tested for spores of *Bacillus larvae* (White), the causative agent of American foulbrood disease. Seven (6.4%) of the colonies tested positive, all with relatively low numbers of spores compared to foragers taken from managed colonies with American foulbrood disease. This suggests that the feral honey bee population in New Zealand may be relatively free of American foulbrood disease and is therefore not a major risk to managed colonies.

**COMMENT: The following two papers are cited by Goodwin et al. 1994 (immediately above)**

Miller, M. E. 1935: Natural comb building. *Canadian Bee Journal* 43: 216-217.

**No abstract is available for this paper; instead a quote from it is given here:**

"There is a bee tree on the main street in our town. This colony has survived three winters, including the winter of 1933-1934, when at least half the colonies maintained under modern conditions in this state were a total loss. [...] This colony of "wild" bees has seen lots of hive colonies in the immediate vicinity abolished by the popular disease. While this is being written these bees are peeking out from their knothole, and thumbing their nose at the bee inspectors. Though the tree is only ten feet from the sidewalk where people pass on the way to church, no one has ever been stung by them. [...] Speaking of bee trees, here is a good one from Michigan. Michigan has, or had, at the date here mentioned, the most drastic bee disease law that the writer has had access to. This is a heavily timbered state, and there are a great many bee trees. These bee trees are "illegal" and are vigorously searched out and destroyed. from the official report of state inspection for the year 1929 we will copy a paragraph: "Bees in trees are considered illegal, and are either killed and sealed up, or removed. The peculiar thing noted last year, that no disease was found in trees, was found to be true the past season; although a large number of colonies of bees in trees were removed and destroyed." During the season of 1929, 13.3 per cent of the regular colonies in the state of Michigan were found to be diseased with A.F.B."

Bailey, L. (1958) Wild honeybees and disease. *Bee World* 39: 93-95.

**As no abstract is available with this paper, a transcript of the entire paper is given here.**

WILD HONEYBEES AND DISEASE by L. BAILEY

Bee Research Department, Rothamsted Experimental Station, Harpenden, England

with acknowledgements to the late E. B. Wedmore, C.B.E, who began the investigation

INTRODUCTION An appeal was made by the Bee Research Association in 1954, through its Progress Reports and *Bee World*, for information about diseases found in wild honeybees (*Apis mellifera*). It was pointed out that there are widely held beliefs either that wild honeybees harbour disease, or that there is a remarkable absence of disease in these bees. Information has been received from various parts of the world as a result of this enquiry, but lack of comparative quantitative data about wild colonies and bee-keepers' colonies in the same area reduces the value of much of it. However, as no further information seems now to be forthcoming, it may be useful to discuss that which is available.

REVIEW OF INFORMATION Professor J. Arth wrote that he had regularly examined bees from 210 wild colonies in the forests of Slavonia (Yugoslavia) between 1920 and 1939. Samples of 100 to 150 bees were examined from half the colonies each spring, and from the other half each autumn. An occasional colony near the periphery of forests was found infected with *Nosema apis*, but usually no parasites of adult bees were found. Beekeepers' colonies which Professor Arth examined were kept in skeps or log hives south of Slavonia in Bosnia-Herzegovina. Of 85 of these colonies, which were examined in one year only, bees in 8 were infected with *Nosema apis* and in 1 with *Acarapis woodi*. This seems a fairly high incidence relative to that in the wild colonies, particularly as the latter were found so free of infection over so many years.

In France, Lieutaud (1954) used swarms from wild colonies to stock his hives near Marseilles. He found no parasites in these bees, although *Nosema apis* and *Acarapis woodi* were present in the few other apiaries in the same district. Moreaux (1954) examined bees from 37 swarms from wild colonies near Nancy and found no parasites. The brood of 9 wild colonies, also near Nancy, was found to be healthy. Unfortunately there is no record of beekeepers' colonies in the same region. In England, G. Wakeford (Sussex) has examined about 100 wild colonies during the past few years and has not yet found a case of foul brood, although this is known to be present locally in beekeepers' colonies. In the U.S.A., Miller (1935) reported that a large number of wild colonies, which were officially condemned in Michigan, were destroyed in 1928 and 1929. No case of foul brood was found, although in the same region over 13 % of beekeepers' colonies were infected with American foul brood. There have been a number of other letters and reports in journals which, although they contain no quantitative information, report the absence of brood diseases in wild colonies or in colonies allowed to run virtually wild ; often disease is said to have been present in the same regions in colonies which had been subjected to the usual beekeeping practices.

Only three instances of brood disease in wild colonies have been reported, two in England and one in Australia. One colony found dead in 1957 in a roof in Dorset by Brigadier C. R. Templer had American foul brood. But records provided by Mr. P. S. Milne of the National Agricultural Advisory Service show that in the same region 38 cases of American foul brood were discovered in 15 different apiaries during 1950 and 1951 ; 16 cases were very near the wild colony, which may therefore have become infected from one of these sources. Another wild colony with American foul brood was found in 1954, also in a roof in Dorset, but the records of the National Agricultural Advisory Service show that there were 18 cases of the disease in the same village at that time. Ryan and Cunningham (1950) reported that one beekeeper had found American foul brood in all the wild colonies (number lot given) that he had taken from trees in a part of Tasmania. But, in the same locality, over half the beekeepers' colonies were infected – 13 colonies out of 15 in one apiary. The possibility that the beekeepers' colonies were the source of such a widespread infection of the wild bees cannot be excluded.

DISCUSSION: No firm general conclusion about disease in wild colonies can be drawn from the evidence given above, but its incidence usually seems to be low relative to that in beekeepers' colonies. There are several possible reasons for this. Probably one of the most important is that combs in uninhabited nests of wild honeybees are quickly destroyed. Professor Arth remarked on the rapid disappearance of combs in uninhabited nests of wild colonies in Slavonia ; Smith (1953) has pointed out that in tropical countries weak colonies are often displaced by wax moths and their combs destroyed. Beekeepers, however, store spare combs and thus tend to preserve those parasites which have resistant stages. The natural method of reproduction of colonies by swarming restricts the transmission of such parasites, but beekeepers often hive swarms and new colonies on preserved combs.

On the whole, beekeepers' colonies are kept closer together than wild colonies would be distributed ; this encourages a higher incidence of robbing and drifting between colonies than would occur under natural conditions, and thereby leads to a higher rate of transmission of parasites between colonies.

In some areas, particularly in the many suburban regions in which forage is scarce, more colonies are kept by beekeepers than could survive naturally. Over-population probably reduces foraging activity and normal growth of individual colonies, and this leads directly to less efficient natural control of disease. It is well known, for example, that European foul brood is rapidly suppressed during good nectar flows ; also strong development of colonies leads to extensive comb cleaning, and to rapid replacement of older bees which will help to suppress infection by *Nosema apis* and *Malpighamoeba mellifica*. Recent work (Bailey, 1958) has shown that high foraging activity suppresses infection with *Acarapis woodi*. There is evidence that even American foul brood may be suppressed by good nectar flows. For example, Ryan and Cunningham (1950) observed the degree of infection with American foul brood in 14 untreated colonies, before and after the only substantial nectar flow in the region. After the flow, infection had diminished in 8 colonies, in 4 it was the same as before, and only in 2 had it increased. In regions of abundant nectar flows, lightly infected colonies may thus be able to suppress the disease effectively; this may help to eliminate American foul brood in highly productive tropical regions (Smith, 1953). But wax moths and other enemies which destroy weak colonies probably play a more important part in eliminating American foul brood in the tropics as a whole.

It may be that most infectious diseases of wild honeybees were restricted originally to bees in regions where nectar flows were unreliable the ecological fringes of suitable habitats ; pathogenic organisms with resistant phases dormant on combs (such as *B. larvae*) may have been confined to the coolest of these ecological

zones where wax moths and other comb scavengers would be less active. If so, beekeepers have certainly extended the range of such diseases.

Suppression of infections by nectar flows suggests that, in the wild, those colonies which develop heaviest infections are likely to be those that have fared worst, and will therefore be most likely to die in winter from shortage of food, as well as from weakening by disease. The primitive beekeepers who killed the heaviest colonies for their honey in autumn (as was found, for example, by Professor Arth), but kept their weaker colonies, would oppose this natural selection of the healthiest colonies; and the common practice today of feeding colonies after a poor season allows many to survive in a heavily parasitized condition.

There seem to be many reasons why beekeepers' colonies should have more disease than wild colonies. It is difficult to formulate such plausible reasons for the opposite point of view, and the evidence so far received indicates that wild colonies are the more healthy.

#### ACKNOWLEDGEMENTS

Sincere thanks are expressed to Professor Arth for his most useful observations, and to all others who kindly sent accounts of their observations and gave useful references.

#### REFERENCES

- BAILEY, L. (1958) The epidemiology of the infestation of the honeybee *Apis mellifera* L. by the mite, *Acarapis woodi* Rennie, and the mortality of infested bees *Parasitology* (in press)
- BEE RESEARCH ASSOCIATION (1954) Wild honeybees and disease *Progr. Rep. No. 21; Bee World* 35(7) : 140
- LIEUTAUD, C. (1954) Abeilles sauvages et maladies *Rev. franc. Apic.* 3(103) : 1000-1001
- MILLER, M. E. (1935) Natural comb building *Canad. Bee J.* 43(8) : 216-217
- MOREAUX, R. (1954) Abeilles sauvages et maladies *Rev. franc. Apic.* 3(102): 961 only
- RYAN, A. F. & CUNNINGHAM, D. G. (1950) Some observations on the control of American foul brood disease of bees by the use of sulphathiazole *Tasm. J. Agric.* 21: 313-317
- SMITH, F. G. (1953) Beekeeping in the tropics *Bee World* 34(12) : 233-245

## Comb with small cell foundation is ineffective against Varroa

COMMENT: For a review of all papers available up to 2011, including those that suggest small cells may be helpful against Varroa please see:

Heaf, D. J. (2011) Do small cells help bees cope with varroa? -- A review. *The Beekeepers Quarterly* (June 2011) No. 104, pp. 39-45.

[http://www.dheaf.plus.com/warrebeekeeping/do\\_small\\_cells\\_help\\_bees\\_cope\\_with\\_varroa.pdf](http://www.dheaf.plus.com/warrebeekeeping/do_small_cells_help_bees_cope_with_varroa.pdf)

Small-cell comb does not control Varroa mites in colonies of honeybees of European origin

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Received 9 July 2010—Revised 26 September 2010—Accepted 4 October 2010

Abstract—

We tested the idea that Varroa destructor can be controlled in colonies of the European subspecies of *Apis mellifera* by providing them with combs built of small cells, in which immature mites might have difficulty developing for lack of space. We established seven pairs of equal-size colonies that started out equally infested with mites. In each pair, one hive contained only standard-cell (5.4 mm) comb, and the other contained only small-cell (4.8 mm) comb. We measured the colonies' mite loads at monthly intervals across a summer. No differences arose between the two treatment groups in their mean mite loads (mites per 100 worker bees or mite drop per 48 h). We suggest that providing small-cell combs did not inhibit mite reproduction because the fill factor (thorax width/cell width) was only slightly higher in the small cells than in the standard cells (79% and 73%, respectively).

*Apidologie* (2011) 42:526–532

A. M. Ellis & G. W. Hayes & J. D. Ellis (2009) The efficacy of small cell foundation as a varroa mite (*Varroa destructor*) control *Exp Appl Acarol* 47:311–316

DOI 10.1007/s10493-008-9221-3

Received: 3 October 2008 / Accepted: 10 November 2008 / Published online: 6 December 2008



Springer Science+Business Media B.V. 2008

#### Abstract

Due to a continuing shift toward reducing/minimizing the use of chemicals in honey bee colonies, we explored the possibility of using small cell foundation as a varroa control. Based on the number of anecdotal reports supporting small cell as an efficacious varroa control tool, we hypothesized that bee colonies housed on combs constructed on small cell foundation would have lower varroa populations and higher adult bee populations and more cm<sup>2</sup> brood. To summarize our results, we found that the use of small cell foundation did not significantly affect cm<sup>2</sup> total brood, total mites per colony, mites per brood cell, or mites per adult bee, but did affect adult bee population for two sampling months. Varroa levels were similar in all colonies throughout the study. We found no evidence that small cell foundation was beneficial with regard to varroa control under the tested conditions in Florida.

DOI: 10.1051/apido/2009049

Jennifer A. Berry<sup>1</sup>, William B. Owens<sup>2</sup> and Keith S. Delaplane<sup>1</sup> (2010) Small-cell comb foundation does not impede Varroa mite population growth in honey bee colonies *Apidologie* **41** 40-44

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Received 1 October 2008 – Revised 23 March 2009 – Accepted 27 April 2009 - Published online 25 September 2009

Abstract - In three independently replicated field studies, we compared biometrics of Varroa mite and honey bee populations in bee colonies housed on one of two brood cell types: small-cell (4.90.08 mm cell width, walls inclusive) or conventional-cell (5.30.04). In one of the studies, ending colony bee population was significantly higher in small-cell colonies (149942494 bees) than conventional-cell 56531082). However, small-cell colonies were significantly higher for mite population in brood (359.787.4 vs. 134.538.7), percentage of mite population in brood (49.47.1 vs. 26.8 6.7), and mites per 100 adult bees (5.10.9 vs. 3.30.5). With the three remaining ending Varroa population metrics, mean trends for small-cell were unfavorable. We conclude that small-cell comb technology does not impede Varroa population growth.

Issue Apidologie

DOI 10.1051/apido/2010003

Published online 04 February 2010

Brood-cell size has no influence on the population dynamics of Varroa destructor mites in the native western honey bee, *Apis mellifera mellifera*\*

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#### Abstract

The varroa mite (*Varroa destructor*) is an ectoparasite of the western honeybee *Apis mellifera* that reproduces in the brood cells. The mite will generally kill colonies unless treatment is given, and this almost universally involves the use of chemicals. This study was undertaken to examine the effect of small cell size on the reproductive success of the mite, as a method of non-chemical control in the Northern European honeybee *Apis mellifera mellifera*. Test colonies with alternating small and standard cell size brood combs were sampled over a three-month period and the population biology of the mites evaluated. To ensure high varroa infestation levels, all colonies were infested with mites from a host colony prior to commencement. A total of 2229 sealed cells were opened and the varroa mite families recorded. While small-sized cells were more likely to be infested than the standard cells, mite intensity and abundance were similar in both cell sizes. Consequently, there is no evidence that small-cell foundation would help to contain the growth of the mite population in honeybee colonies and hence its use as a control method would not be proposed.

Journal of Apicultural Research and Bee World 47(4): 239–242 (2008)

The effect of honey bee worker brood cell size on Varroa destructor infestation and reproduction.

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Received 30 April 2007, accepted subject to revision 22 July 2007, accepted for publication 11 August 2008.

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#### Summary

The effect of honey bee (*Apis mellifera*) worker brood cell size on cell infestation and reproductive success of *Varroa destructor* in New Zealand was determined by establishing ten nucleus colonies with mosaic frames, each consisting of cells drawn from five different foundation sizes. When the brood were 18–20 days old, 1636 cells were individually uncapped and the number of adult and deutonymph female mites were recorded. The internal width of each brood cell was also measured. The data were analysed according to the imprint size of the “foundation” specified by the supplier, and the “measured” internal width of each individual drawn brood cell. The “foundation” cell size had no significant effect on the reproductive success of *V. destructor*, but the proportion of cells that were infested by adult female mites was significantly different. A significantly higher proportion of the cells drawn from the 4.8 mm imprint “foundation” were infested compared to those of the other sizes. “Measured” brood cell size had no significant effect on mite reproduction or infestation.

DOI: 10.3896/IBRA.1.47.4.01

Small cells -- a possible way out of the *Varroa* crisis?

Harald Singer, Austria in 'Bienen Aktuell 04/2007'

In a research project, *Varroa* infestation with a cell sizes of 4.9 mm was compared with that of 5.9 mm. After analysing the data obtained, it was possible to establish a trend in favour of the smaller cells. However, in addition to this planned analysis, other influencing factors were revealed, such as location, year, queen genetics, all of which can have equally significantly lowered *Varroa* reproduction rates in spite of larger cells. Of 22 locations, 6 locations were significant in relation to *Varroa* pressure and to the build up of the *Varroa* populations. The colonies in these locations simply had fewer mites, regardless of the larger or smaller cells. The particular year also had a very broad influence on the development of mite populations. In any case, it is not yet possible to confirm the expectation that bees that build on 4.9 mm cell foundation generally show a lower reproductive rate.

## Mesh floors – pros and cons as regards *Varroa* elimination

Rinderer T.E., de Guzman L.I., Delatte G.T., Harper C. (2003) An evaluation of ARS Russian honey bees in combination with other methods for the control of *Varroa* mites, *Am. Bee J.* 143, 410–413

#### Abstract

The effects of several management tools for the control of *Varroa destructor* were evaluated. Both domestic and Russian honey bee (ARS Primorsky) colonies were provided with: 1) a single application of formic acid in gel formulation in hives with solid bottom boards, 2) a single application of formic acid in gel formulation in hives with screened bottom boards, 3) no chemical treatment to hives with screened bottom boards, or 4) no chemical treatment to hives with solid bottom boards. Overall, Russian honey bees had about half the number of *V. destructor* found in domestic colonies at the end of the experiments. Formic acid reduced the numbers of mites in colonies regardless of the type of bottom board. Screened bottom boards did not reduce the number of mites in colonies. The combination that resulted in the fewest number of mites was Russian colonies in hives that were treated with formic acid.

[http://www.russianbreeder.org/uploads/1/0/3/2/10328665/407-rinderer--an\\_evaluation\\_of\\_ars.pdf](http://www.russianbreeder.org/uploads/1/0/3/2/10328665/407-rinderer--an_evaluation_of_ars.pdf)

*Journal of Apicultural Research* 43(3): 114–117(2004) © IBRA 2004

Effect of screen floors on populations of honey bees and parasitic mites (*Varroa destructor*)

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Received 30 January 2004, accepted subject to revision 5 March 2004, accepted for publication 25 June 2004

#### SUMMARY

This study compared brood production, honey consumption (in winter only), population growth of honey bees (*Apis mellifera*), and population growth of parasitic mites (*Varroa destructor*) in hives with open screen

or wood as floor material. Two experiments were conducted in Baton Rouge, Louisiana, USA, one in winter (19 colonies) and one in summer (22 colonies). In both experiments, we established uniform colonies of honey bees by subdividing 30 kg of mite-infested bees. Each colony began with about 11000 bees, no brood, and uniform populations of mites (127 and 480 mites per colony in winter and summer, respectively). The summer test included a third treatment (8 colonies) where a wooden tray (5 cm deep) closed the space beneath a screen floor. After the first 20 days of the experiments, when no adult bees or mites had yet been produced in any of the colonies, the treatments showed no differences in brood production, honey consumption, or survival of adult bees. At nine weeks, colonies with screen floors had fewer mites, a lower percentage of their mite population residing in brood cells (open screen only), and more cells of capped brood. These results suggest that colonies with open-screen floors may hold back the growth of mite populations by decreasing the rate at which mites invade brood cells.

**COMMENT: The following paper would count agaisnd *open* mesh floors.**

High Humidity in the Honey Bee (*Apis mellifera* L.) Brood Nest Limits Reproduction of the Parasitic Mite *Varroa jacobsoni* Oud.

B. Kraus, H.H.W. Velthuis

Kraus, B. & Velthuis, H.H.W. (1997) High Humidity in the Honey Bee (*Apis mellifera* L.) Brood Nest Limits Reproduction of the Parasitic Mite *Varroa jacobsoni* Oud. *Naturwissenschaften* **84** 217–218

Universiteit Utrecht, Vakgroep Vergelijkende Fysiologie,

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*Naturwissenschaften* 84, 217–218 (1997) © Springer-Verlag 1997

No abstract, but concluding paragraph:

The present data provide a simple explanation for the fact that often in tropical climates population growth of *V. jacobsoni* is unexpectedly low. The significant impact of differences in relative humidity of only 9–25% upon reproduction and therewith on population growth of a parasite demonstrates another probable effect of environmental factors upon parasite virulence.

**COMMENT: The following paper is not from a peer reviewed journal but is a government report, and appears to involve some careful experimentation..**

Experimentation of an Anti-Varroa Screened Bottom Board in the Context of Developing an Integrated Pest Management Strategy for Varroa Infested Honeybees in the Province of Quebec accomplished within the framework of the program:

“Appui au développement de l’agriculture et de l’agroalimentaire en région 2000-2003” of the “Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec, Canada (Regional district of l’Estrie)

Final Report by Jean Pierre Chapleau,

March 2002 translated and revised March 2003

<http://www.apinovar.com/articles/AV-BOTTOM%20BOARD.pdf>

The anti-varroa screened bottom board was tested on a large scale during the beekeeping seasons of 2000 and 2001 in the l’Estrie region of Quebec. Used with its bottom closed by a sampling drawer, this bottom board succeeded in reducing, on average, by 37% the varroa populations of the colonies during the season of 2001. The global results obtained however were not statistically significant except for certain sampled sub groups where the experiment conditions were more homogeneous. These results reinforce the conclusions drawn from two recent studies performed in the United States that were also statistically non significant. A 14 months comparison by T.C Webster posterior to our work showed a 70% highly significant reduction of the varroa population (17) with the screened bottom. The performance of the bottom board varied according to the apiary sites and it is possible that certain environmental factors affected its efficiency. More research is necessary to better comprehend this aspect. The antivarroa bottom board must never be used with its bottom hole opened as this leads to a lowering of cluster temperature resulting in ideal conditions for varroa development. As confirmed in 2000, this situation not only negated the beneficial effects of the bottom board, it also resulted in a net increase in the mite infestation rate (29.2% more varroa mites, non significant) as compared to the control group. The performance of the bottom board also varied from one colony to

another. It was observed that the strength of the colony in the spring and especially the lineage of the queen were significant factors in the rate of infestation. The anti-varroa screened bottom board appears to also increase the effectiveness of varroacidal treatments and its use could delay the development of mite resistance to chemical medications. The anti-varroa screened bottom board is readily modified to include a removable drawer for sampling purposes. The bottom boards used in our tests were thus equipped with removable trays which greatly simplified screening and enabled us to use natural varroa drop over prolonged periods of time as indicators of the level of infestation of colonies. This advantage is a bonus, specially during periods of nectar flow. The use of the anti-varroa screened bottom board is an easy, economical, long lasting and environmentally friendly method to combat the varroa mite. It constitutes, in our opinion, an indispensable tool in an integrated pest management strategy not only because it contributes to slowing down the rate of infestation but also because we can easily determine, at any time, the level of infestation. It then becomes an important tool for decision making, re choosing control methods and the timing of applications. It can also be used in conjunction with other methods of control such as the use of resistant queens and the punctual application of essential oils or formic acid. With its use, dependence on chemical treatments can be greatly reduced if not eliminated. Such a set-up could probably constitute an adequate integrated pest management strategy. The anti-varroa screened bottom board promotes the natural grooming behavior of the honeybee. The process of selection could further develop this behavior. The screened bottom board also presents an opportunity for a different perspective on developing control methods that would simply consist of causing the mites to drop off of adult bees.

Title: Overwintering of Russian Honey Bees in Northeastern Iowa

Authors: Villa, Joseph Rinderer, Thomas Bigalk, Manley - GOLDEN RIDGE HONEY FARMS

Submitted to: Bee Culture

Publication Type: Peer Reviewed Journal

Publication Acceptance Date: April 1, 2009

Publication Date: May 1, 2009

Citation: Villa, J.D., Rinderer, T.E., Bigalk, M. 2009. Overwintering of Russian Honey Bees in Northeastern Iowa. *Science of Bee Culture* 1(2):19-21; supplement to *Bee Culture* 137(2).

Technical Abstract: The survival, tracheal mite infestation, worker population and weight loss of colonies from Russian test lines were evaluated during three winters (2001, 2002 and 2003) in Cresco, Iowa. Overall, 90% of the colonies survived the period from November to April. The percentage of bees infested with tracheal mites in most Russian colonies in August, November and April were below the economic threshold of 20%. Surviving Russian colonies had good populations at the end of the overwintering period. Colony weight loss from November to April was on average less than 20 lbs. The use of a screened bottom board increased weight loss by 20% compared to a standard wooden bottom board. Russian bees provide a viable alternative for beekeepers needing to overwinter colonies in northern states.

M. Keshlaf, R. Spooner-Hart

Effects of Modified Bottom Boards on the Performance of Honeybee Colonies

*International Journal of Biological, Veterinary, Agricultural and Food Engineering* Vol:8 No:1, 2014, pp. 25-28

Abstract—

Australia does not have varroa mite. However, we investigated whether modified hive bottom boards used for varroa mite management in honey bee colonies had other benefits, for honey production. We compared a number of colony parameters between hives fitted with tube, mesh and conventional (solid) bottom boards in two locations in eastern Australian, Richmond NSW and Castlemaine Victoria. Colonies housed in hives with mesh and tube bottom boards were not significantly superior to those in hives with conventional bottom boards with regard to bee flight activity, nor did they produce more honey, brood or stored pollen, in either experimental site. Although the trial was conducted over only one season, it is suggested that there may be no benefit in Australian bee keepers changing from using conventional bottom boards in the absence of varroamite.

## **Queen production, mating, artificial insemination and physiology**

Context dependent bias in honeybee queen selection: swarm versus emergency queens

H. Michael G. Lattorff<sup>1,2</sup> & Robin F. A. Moritz<sup>1,2</sup>

Behav Ecol Sociobiol DOI 10.1007/s00265-016-2151-x

Received: 28 January 2016 / Revised: 28 April 2016 / Accepted: 10 May 2016

#Springer-Verlag Berlin Heidelberg 2016

Abstract

Colonies of social Hymenoptera with multiple mated queens comprise of numerous super-sister subfamilies with high within but low between subfamily relatedness. Because fitness gain in workers is indirect and relatedness dependent, inclusive fitness theory predicts that nepotism might be adaptive, particularly with respect to queen rearing and selection of new queens. However, nepotistic queen rearing could not be detected in various eusocial insect species and instead workers preferentially rear queens from rare subfamilies after queen loss. Here, we test, whether this bias can also be observed in queen rearing in preparation for reproductive swarming in the honeybee, *Apis mellifera*. Colonies reared swarm and subsequently emergency queens. Genotyping of all produced queens as well as worker brood revealed a significant deviation from the expected worker subfamily distribution for emergency queens but not for swarm queens. The preference of rare subfamilies may result from worker control over queen rearing in emergency queen rearing. Based on our results it appears that workers choose a few out of a vast number of worker-destined larvae to select emergency queens at extreme selection intensity. This sets the stage for competition among larvae to compete for queen caste fate, presumably by differentially attracting nurse workers to raise them as queens. During swarm queen production, workers cannot select larvae, because the queen lays eggs into predestined cells, resulting in subfamily distributions among the queens more similar to a random subfamily sample as determined in the worker brood.

Naturwissenschaften (2005) 00

DOI 10.1007/s00114-005-0025-6

SHORT COMMUNICATION

Robin F. A. Moritz · H. Michael G. Lattorff · Peter Neumann F. Bernhard Kraus Sarah E. Radloff H. Randall Hepburn

Rare royal families in honeybees, *Apis mellifera*

Abstract The queen is the dominant female in the honeybee colony, *Apis mellifera*, and controls reproduction. Queen larvae are selected by the workers and are fed a special diet (royal jelly), which determines caste. Because queens mate with many males a large number of subfamilies coexist in the colony. As a consequence, there is a considerable potential for conflict among the subfamilies over queen rearing. Here we show that honeybee queens are not reared at random but are preferentially reared from rare “royal” subfamilies, which have extremely low frequencies in the colony’s worker force but a high frequency in the queens reared.

Honey bee colonies regulate queen reproductive traits by controlling which queens survive to adulthood

D. R. Tarpay<sup>1,2</sup> M. Simone-Finstrom<sup>1,2,3,4</sup> T. A. Linksvayer<sup>3</sup>

Insect. Soc. DOI 10.1007/s00040-015-0452-0 Insectes Sociaux

Abstract The production of new queens in honey bee colonies is one of the most important determinants of reproductive success, and it involves cooperative behavior among hundreds or thousands of workers. Colony members are generally expected to benefit by optimizing the reproductive traits of prospective replacement queens, but potential conflicts of interest among colony members could result in suboptimal queens. We studied the degree to which colonies regulate adult queen traits by controlling access to developing queens that survived from pupation to adulthood. We also searched for evidence of strong conflict among patriline by comparing the contribution of patriline to new queens and new workers, although we found no evidence for the existence of significantly queen-biased patriline or for any association between patriline contribution to new queens and queen traits. However, adult queens emerging from cells accessible to workers were larger in terms of compared to adult queens emerging from cells that were not accessible to workers. These results suggest that colonies regulate queen quality traits by curtailing low-quality queens from fully developing, which is further evidence that cooperation predominates over potential conflict within honey bee colonies.

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Behavioral Ecology Volume 20, Number 5 Pp. 1007-1014

Queen reproductive state modulates pheromone production and queen-worker interactions in honeybees  
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Kochera, S. D., Richard, F.-J., Tarpy, D. R. Grozinger, C. M. Queen reproductive state modulates pheromone production and queen-worker interactions in honeybees. *Behavioral Ecology* **20**(5) 1007-1014

Abstract

The mandibular glands of queen honeybees produce a pheromone that modulates many aspects of worker honeybee physiology and behavior and is critical for colony social organization. The exact chemical blend produced by the queen differs between virgin and mated, laying queens. Here, we investigate the role of mating and reproductive state on queen pheromone production and worker responses. Virgin queens, naturally mated queens, and queens instrumentally inseminated with either semen or saline were collected 2 days after mating or insemination. Naturally mated queens had the most activated ovaries and the most distinct chemical profile in their mandibular glands. Instrumentally inseminated queens were intermediate between virgins and naturally mated queens for both ovary activation and chemical profiles. There were no significant differences between semen- and saline-inseminated queens. Workers were preferentially attracted to the mandibular gland extracts from queens with significantly more activated ovaries. These studies suggest that the queen pheromone blend is modulated by the reproductive status of the queens, and workers can detect these subtle differences and are more responsive to queens with higher reproductive potential. Furthermore, it appears as if insemination substance does not strongly affect physiological characteristics of honeybee queens 2 days after insemination, suggesting that the insemination process or volume is responsible for stimulating these early postmating changes in honeybee queens.

Key words: behavior, caste, chemical communication, pheromone, reproduction, social insect.

Website: <http://entomology.ncsu.edu/grozinger>

Effects of Insemination Quantity on Honey Bee Queen Physiology

Last Updated: July 20, 2009 Related resource areas: Bee Health

Citation: Richard F-J, Tarpy DR, Grozinger CM (2007) Effects of Insemination Quantity on Honey Bee Queen Physiology. *PLoS ONE* **2**(10): e980. doi:10.1371/journal.pone.0000980

Web Link: [PLoSone article](#)

Brief Description: Mating has profound effects on the physiology and behavior of female insects, and in honey bee (*Apis mellifera*) queens, these changes are permanent. Queens mate with multiple males during a brief period in their early adult lives, and shortly thereafter they initiate egg-laying. Furthermore, the pheromone profiles of mated queens differ from those of virgins, and these pheromones regulate many different aspects of worker behavior and colony organization. While it is clear that mating causes dramatic changes in queens, it is unclear if mating number has more subtle effects on queen physiology or queen-worker interactions; indeed, the effect of multiple matings on female insect physiology has not been broadly addressed. Because it is not possible to control the natural mating behavior of queens, we used instrumental insemination and compared queens inseminated with semen from either a single drone (single-drone inseminated, or SDI) or 10 drones (multi-drone inseminated, or MDI). We used observation hives to monitor attraction of workers to SDI or MDI queens in colonies, and cage studies to monitor the attraction of workers to virgin, SDI, and MDI queen mandibular gland extracts (the main source of queen pheromone). The chemical profiles of the mandibular glands of virgin, SDI, and MDI queens were characterized using GC-MS. Finally, we measured brain expression levels in SDI and MDI queens of a gene associated with phototaxis in worker honey bees (*Amfor*).

Implications: Here, we demonstrate for the first time that insemination quantity significantly affects mandibular gland chemical profiles, queen-worker interactions, and brain gene expression. Further research will be necessary to elucidate the mechanistic bases for these effects: insemination volume, sperm and seminal protein quantity, and genetic diversity of the sperm may all be important factors contributing to this profound change in honey bee queen physiology, queen behavior, and social interactions in the colony.

Tarpy DR, Delaney DA, Seeley TD (2015)

Mating Frequencies of Honey Bee Queens (*Apis mellifera*L.) in a Population of Feral Colonies in the Northeastern United States.

PLoS ONE 10(3): e0118734. doi:10.1371/journal.pone.0118734

Abstract

Across their introduced range in North America, populations of feral honey bee (*Apis mellifera*L.) colonies have supposedly declined in recent decades as a result of exotic parasites, most notably the ectoparasitic mite *Varroa destructor*. Nonetheless, recent studies have documented several wild populations of colonies that have persisted. The extreme polyandry of honey bee queens—and the increased intracolony genetic diversity it confers—has been attributed, in part, to improved disease resistance and may be a factor in the survival of these populations of feral colonies. We estimated the mating frequencies of queens in feral colonies in the Arnot Forest in New York State to determine if the level of polyandry of these queens is especially high and so might contribute to their survival success. We genotyped the worker offspring from 10 feral colonies in the Arnot Forest of upstate New York, as well as those from 20 managed colonies closest to this forest. We found no significant differences in mean mating frequency between the feral and managed queens, suggesting that queens in the remote, low-density population of colonies in the Arnot Forest are neither mate-limited nor adapted to mate at an especially high frequency. These findings support the hypothesis that the hyperpolyandry of honey bees has been shaped on an evolutionary timescale rather than on an ecological one.

Development of the Honey Bee Gut Microbiome throughout the Queen-Rearing Process

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Appl. Environ. Microbiol. May 2015 vol. 81 no. 9 3182-3191

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The European honey bee (*Apis mellifera*) is used extensively to produce hive products and for crop pollination, but pervasive concerns about colony health and population decline have sparked an interest in the microbial communities that are associated with these important insects. Currently, only the microbiome of workers has been characterized, while little to nothing is known about the bacterial communities that are associated with queens, even though their health and proper function is central to colony productivity. Here, we provide a large-scale analysis of the gut microbiome of honey bee queens during their developmental trajectory and through the multiple colonies that host them as part of modern queen-rearing practices. We found that queen microbiomes underwent a dramatic shift in size and composition as they aged and encountered different worker populations and colony environments. Queen microbiomes were dominated by enteric bacteria in early life but were comprised primarily of alphaproteobacteria at maturity. Furthermore, queen gut microbiomes did not reflect those of the workers who tended them and, indeed, they lacked many of the bacteria that are considered vital to workers. While worker gut microbiotas were consistent across the unrelated colony populations sampled, the microbiotas of the related queens were highly variable. Bacterial communities in mature queen guts were similar in size to those of mature workers and were characterized by dominant and specific alphaproteobacterial strains known to be associated with worker hypopharyngeal glands. Our results suggest a model in which queen guts are colonized by bacteria from workers' glands, in contrast to routes of maternal inoculation for other animal microbiomes.

COMMENT: The following text is quoted from the above paper:

"In contrast, virtually nothing is known about the microbial communities that are associated with queens, even though their health and proper function is central to the productivity of their colonies. Complicating such studies are the realities of queen production in modern apiculture, where colonies are managed

intensively to yield hive products (e.g., honey) and particularly for crop pollination. In managed colonies, the natural process of queen replacement, where an aging queen is superseded by a daughter queen, raised by her worker sisters from within the larval ranks, typically is prevented by beekeepers. Instead, intentionally bred queens are artificially introduced to a colony by beekeepers only after taking a circuitous path through several other related and unrelated host colonies."

Journal of Apicultural Research 53(3): 337-363 (2014)

DOI 10.3896/IBRA.1.53.3.02

A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies

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#### Summary

The term "quality" in relation to queens and drones refers to certain quantitative physical and / or behavioural characters. It is generally believed that a high quality queen should have the following physical characteristics: high live weight; high number of ovarioles; large size of spermatheca; high number of spermatozoa in spermatheca; and be free from diseases and pests. It is, however, also known that the performance of a honey bee colony is the result of its queen's function as well as of that of the drones that mated with her. These two approaches are often considered together and give a general picture of the queen production technique and selection. Here we describe the most common and well known anatomical, physiological, behavioural and performance characters related to the queens, as measured in different European countries: the live weight of the virgin queen (Bulgaria); the live weight of the laying queen (Bulgaria, Italy); the diameter and volume of spermatheca (Bulgaria, Greece, Slovenia); the number of ovarioles (Greece, Italy, Slovenia); the weight of ovaries (Slovenia); the number of spermatozoa in spermatheca (Italy, Poland, Slovenia); the brood pattern (Bulgaria, Greece); the egg laying ability / fecundity (Bulgaria); the brood production (Croatia, Serbia); the colony population development (Croatia, Serbia, Slovakia); the honey production (Croatia, Denmark, Serbia, Slovakia); the hygienic behaviour (Croatia, Denmark, Serbia, Slovakia); the defence behaviour (Croatia); the calmness / sitting on the comb (Croatia, Denmark); and swarming (Croatia, Denmark). The data presented fit well with the findings of the same characters in the literature, and in general they support the argument for the term "quality characters". Especially for the weight of the queen, the number of ovarioles, the volume of the spermatheca and the number of spermatozoa, data per country proved its own accuracy by repetition through the years. We also report that when instrumentally inseminated queens are kept under mass production conditions (in small cages in queen banks and with low number of attendants) they can transfer the semen to their spermatheca and clear their oviducts more efficiently when they have been inseminated with small amounts of semen in



two or three sequences (but not four), compared to those inseminated with the same amount of semen at once (Poland). Furthermore, we had an inside view of the sanitary conditions of the colony: a. through the health status of the queen (nosema plus virus analysis) (Slovenia); and b. evaluating the nosema load of worker bees (Denmark) and of the queens (Greece). This is the first step to summarize this type of diverse data for such an important issue. The knowledge acquired can be used to fill in the existing gaps in the breeding or queen evaluation systems of each country in order to facilitate standardization of methodology for comparable results.

*Insectes sociaux*. 46 (1999) 372 – 377

Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality

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Received 21 September 1998; revised 19 January 1999; accepted 9 March 1999.

Summary.

The requeening process was investigated under emergency conditions in honey bee colonies (*Apis mellifera* L.). The progression of queen cell construction was closely monitored after removal of the mother queen, and the newly emerged queens were measured for several physical traits to quantify their reproductive potential (= quality). The results suggest that workers regulate the queen rearing process by differentially constructing cells. Workers built different numbers of queens cells from different ages of brood and non-randomly destroyed over half (53 %) of the initiated cells before their emergence. For those queens whose cells were not torn down, the variation in reproductive quality was limited, varying only slightly among age groups for queen size. Several hypotheses are discussed which might explain the adaptive benefit of worker regulation during queen rearing.

COMMENT: The following paper and a number of others indicate that commercial queen quality is generally satisfactory. However, most of the research looks at a limited number of isolated characteristics and is unable to assess long-term survival of colonies, especially in management systems where regular artificial requeening is practised.

*Apidologie* (2011) 42:1–13

The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.)\*

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Received 23 October 2009 – Revised 26 January 2010 – Accepted 3 February 2010

Abstract–

Understanding the reproductive potential (“quality”) of queens bees can provide valuable insights into factors that influence colony phenotype. We assayed queens from various commercial sources for various measures of potential queen quality, including their physical characters (such as their degree of parasitism), insemination number (stored sperm counts), and effective paternity frequency (number of drone fathers among their offspring). We found significant variation in the physical, insemination, and mating quality of commercially produced queens, and we detected significant correlations within and among these various measures. Overall, the queens were sufficiently inseminated ( $3.99 \pm 1.504$  million sperm) and mated with an appropriate number of drones (effective paternity frequency:  $16.0 \pm 9.48$ ). Importantly, very few of the queens were parasitized by tracheal mites and none were found with either *Nosema* species. These findings suggest possible mechanisms for assessing the potential fitness of honey bee queens without the need for destructive sampling.

Assessing the Mating ‘Health’ of Commercial Honey Bee Queens

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*J. Econ. Entomol.* 105(1): 20–25 (2012); DOI: <http://dx.doi.org/10.1603/EC11276>

ABSTRACT Honey bee queens mate with multiple males, which increases the total genetic diversity

within colonies and has been shown to confer numerous benefits for colony health and productivity. Recent surveys of beekeepers have suggested that poor queens are a top management concern, thus investigating the reproductive quality and mating success of commercially produced honey bee queens is warranted. We purchased 80 commercially produced queens from large queen breeders in California and measured them for their physical size (fresh weight and thorax width), insemination success (stored sperm counts and sperm viability), and mating number (determined by patriline genotyping of worker offspring). We found that queens had an average of  $4.37 \pm 1.446$  million stored sperm in their spermathecae with an average viability of  $83.7 \pm 13.33\%$ . We also found that the tested queens had mated with a high number of drones (average effective paternity frequency:  $17.0 \pm 8.98$ ). Queen "quality" significantly varied among commercial sources for physical characters but not for mating characters. These findings suggest that it may be more effective to improve overall queen reproductive potential by culling lower-quality queens rather than systematically altering current queen production practices.

The effects of mating and instrumental insemination on queen honey bee flight behaviour and gene expression

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Abstract

Mating is fundamental to most organisms, although the physiological and transcriptional changes associated with this process have been largely characterized only in *Drosophila melanogaster*. In this study, we use honey bees as a model system because their queens undergo massive and permanent physiological and behavioural changes following mating. Previous studies have identified changes associated with the transition from a virgin queen to a fully mated, egg laying queen. Here, we further uncouple the mating process to examine the effects of natural mating vs. instrumental insemination and saline vs. semen insemination. We observed effects on flight behaviour, vitellogenin expression and significant overlap in transcriptional profiles between our study and analogous studies in *D. melanogaster*, suggesting that some post-mating mechanisms are conserved across insect orders.

*Insect Molecular Biology* (2010) 19(2), 153–162 doi: 10.1111/j.1365-2583.2009.00965.x

*Apidologie* 40 (2009) 464-471

DOI: 10.1051/apido/2009008

Comparisons of the queen volatile compounds of instrumentally inseminated versus naturally mated honey bee (*Apis mellifera*) queens

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Abstract -

Instrumental insemination is an attractive alternative to natural mating because specific genetic crosses can be made, thus producing colonies with desired traits. However, there are conflicting reports on the quality and acceptance of instrumentally inseminated (II) queens compared to naturally mated (NM) queens. One factor that affects acceptance and retention of queens is the volatile compounds they produce. Our study compared volatile chemicals from virgin and mated honey bee queens that were either NM or II. The volatile compounds from virgin queens differed from those of mated egg-laying queens. Virgin queens produced greater relative amounts of the volatile compounds we detected (including 2-phenylethanol, n-octanal, and n-decanal) with the exception of E- $\alpha$ -ocimene, which was higher in mated laying queens. II and NM queens did not differ in type or relative amounts of volatile compounds. The similarities between II and NM queens indicate that the physiological changes that happen after mating and egg laying occur regardless of the mating method.

Pettis JS, Rice N, Joselow K, vanEngelsdorp D, Chaimanee V (2016) Colony Failure Linked to Low Sperm Viability in Honey Bee (*Apis mellifera*) Queens and an Exploration of Potential Causative Factors.

PLoS ONE 11(2): e0147220. doi:10.1371/journal.pone.0147220

Queen health is closely linked to colony performance in honey bees as a single queen is normally responsible for all egg laying and brood production within the colony. In the U. S. in recent years, queens have been failing at a high rate; with 50% or greater of queens replaced in colonies within 6 months when historically a queen might live one to two years. This high rate of queen failure coincides with the high mortality rates of colonies in the US, some years with >50% of colonies dying. In the current study, surveys of sperm viability in US queens were made to determine if sperm viability plays a role in queen or colony failure. Wide variation was observed in sperm viability from four sets of queens removed from colonies that beekeepers rated as in good health (n = 12; average viability = 92%), were replacing as part of normal management (n = 28; 57%), or where rated as failing (n = 18 and 19; 54% and 55%). Two additional paired set of queens showed a statistically significant difference in viability between colonies rated by the beekeeper as failing or in good health from the same apiaries. Queens removed from colonies rated in good health averaged high viability (ca. 85%) while those rated as failing or in poor health had significantly lower viability (ca. 50%). Thus low sperm viability was indicative of, or linked to, colony performance. To explore the source of low sperm viability, six commercial queen breeders were surveyed and wide variation in viability (range 60–90%) was documented between breeders. This variability could originate from the drones the queens mate with or temperature extremes that queens are exposed to during shipment. The role of shipping temperature as a possible explanation for low sperm viability was explored. We documented that during shipment queens are exposed to temperature spikes (<8 and >40°C) and these spikes can kill 50% or more of the sperm stored in queen spermathecae in live queens. Clearly low sperm viability is linked to colony performance and laboratory and field data provide evidence that temperature extremes are a potential causative factor.

<http://link.springer.com/article/10.1007/s00040-016-0537-4>

Insectes Sociaux pp 1-14 First Online: 01 February 2017. DOI: 10.1007/s00040-016-0537-4

Levels of selection shaping caste interactions during queen replacement in the honey bee, *Apis mellifera*  
K. Long, T. T. Cao, J. J. Keller, D. R. Tarpy, M. Shin, S. S. Schneider

We investigated the different levels of selection that may influence the queen replacement process in honey bees by monitoring queen-queen and worker-queen interactions in queenless observation colonies containing populations of paint-marked workers that were either related or not related to introduced virgin queens experimentally reared to be of low- and high-quality, as estimated by differences in size and potential reproductive capacities. The high-quality queens were more likely to survive and become the new laying queen of their colonies. Queen survival was positively associated with both queen fighting ability and worker-queen interactions. Surviving queens were more aggressive and had greater fighting success. However, surviving queens were not bigger than killed queens nor were they the first queens to emerge, suggesting that queen size and early emergence alone are not the determining factors of queen fighting ability. The worker-queen interaction that was most strongly associated with the outcome of queen replacement was the vibration signal, which is a communication signal that workers perform on virgin queens. Surviving queens were vibrated at rates 3-4 times those experienced by killed queens and a queen's vibration rate was positively correlated with her fighting ability and the number of rivals killed. Workers showed no consistent preferences for related vs. unrelated queens and the proportion of interactions received from related workers was not associated with any aspect of queen fighting ability and success monitored. Our results suggests that caste interactions during queen replacement have been shaped by both selection acting at the level of the individual queen (which favors higher quality queens with greater fighting ability) and selection acting at the level of the colony (which favors workers directing vibration signals towards queens with greater fighting potential), ultimately resulting in a higher quality queen becoming the new laying queen of the colony. Selection acting at the level of the individual worker through kin selection, which would favor preferential treatment of related queens, did not consistently influence caste interactions or the outcome of the replacement process. Thus, the outcome of queen replacement in honey bees may be determined primarily by a combination of a queen's inherent fighting ability coupled with the rate at which she receives some interactions (particularly vibration signals) from workers.

## Queen breeding, genetic diversity and local adaptation

Anton Imdorf, Kaspar Ruoff, Peter Fluri ALP forum 2008, Nr. 68 d  
VOLKSENTWICKLUNG BEI DER HONIGBIENE

Swiss Bee Research Centre: Agroscope Liebefeld-Posieux ALP

**COMMENT: No abstract is available for this 86-page publication, but in it we find the following:**

*Abb. 18: Zucht und Volksentwicklung*

*Die Volksentwicklung von sieben vorselektionierten Zuchtlinien aus deutschen Bieneninstituten und einer züchterisch unbearbeiteten Herkunft wurde untersucht. Die Völker waren auf drei Standorte mit sehr ähnlichen klimatischen Bedingungen und Trachtverhältnissen verteilt. Zwischen den untersuchten Völkergruppen konnte weder bei der Volksentwicklung noch beim Honigertrag ein signifikanter Unterschied festgestellt werden (Aumeier und Böcking, unveröffentlichte Resultate).*

### **TRANSLATION**

Fig. 18: Breeding and colony population development

Colony population development of seven preselected breeders' queen lines from German Bee Research Institutes and a queen source not worked on by breeders were investigated. The colonies were distributed between three apiary locations having very similar climatic and foraging conditions. No significant differences were observed either in colony population development or in honey yield. (unpublished findings Aumaier and Böcking). (Page 21)

REVIEW ARTICLE: Conserving diversity and vitality for honey bee breeding

Marina D Meixner<sup>1\*</sup>, Cecilia Costa<sup>2</sup>, Per Kryger<sup>3</sup>, Fani Hatjina<sup>4</sup>, Maria Bouga<sup>5</sup>, Evgeniya Ivanova<sup>6</sup>,  
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Meixner, M. D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E. & Büchler, R. (2010) Conserving diversity and vitality for honey bee breeding. *J. Apicultural Res.* **49**(1) 85-92

### Summary

Beekeepers in Europe, North America and other parts of the world have repeatedly been afflicted by elevated and sometimes unexplained colony losses. Multiple factors have been considered in connection with increased winter losses. In addition to national programmes investigating possible causes for increased honey bee mortality, scientists collaborate at an international level on different aspects of bee health within the COLOSS network. Within this network, Working Group 4 explores aspects of genetic diversity in relation to the vitality and health of honey bee populations. In this paper, we briefly review the genetic diversity of honey bees in Europe, discuss the effects of beekeeping and selective breeding on honey bee populations under the aspect of genetic diversity and bee health, and review the current status of EU legislation with respect to protection of native bee populations. We introduce and discuss recent approaches in honey bee selective breeding to improve disease resistance by introducing traits related to colony vitality. Finally, we present the aims of WG4 within the COLOSS network and briefly introduce our experimental approach.

Journal of Apicultural Research 49(1): 85-92 (2010) © IBRA 2010

DOI 10.3896/IBRA.1.49.1.12

Management increases genetic diversity of honey bees via admixture

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Abstract

The process of domestication often brings about profound changes in levels of genetic variation in animals and plants. The honey bee, *Apis mellifera*, has been managed by humans for centuries for both honey and wax production and crop pollination. Human management and selective breeding are believed to have caused reductions in genetic diversity in honey bee populations, thereby contributing to the global declines threatening this ecologically and economically important insect. However, previous studies supporting this claim mostly relied on population genetic comparisons of European and African (or Africanized) honey bee races; such conclusions require reassessment given recent evidence demonstrating that the honey bee originated in Africa and colonized Europe via two independent expansions. We sampled honey bee workers from two managed populations in North America and Europe as well as several old-world progenitor populations in Africa, East and West Europe. Managed bees had highly introgressed genomes representing admixture between East and West European progenitor populations. We found that managed honey bees actually have higher levels of genetic diversity compared with their progenitors in East and West Europe, providing an unusual example whereby human management increases genetic diversity by promoting admixture. The relationship between genetic diversity and honey bee declines is tenuous given that managed bees have more genetic diversity than their progenitors and many viable domesticated animals. Keywords: admixture, *Apis mellifera*, breeding, management

Received 23 November 2011; revision received 14 March 2012; accepted 23 March 2012

Molecular Ecology (2012) doi: 10.1111/j.1365-294X.2012.05614.x

**COMMENT: The following item is a poster by the authors published 2010 or later.**

Roxane M. Magnus, Amber D. Tripodi, and Allen L. Szalanski

University of Arkansas, Department of Entomology, Fayetteville, AR

Mitochondrial DNA Variation of Queen Breeder and Feral Honey Bee

(*Apis mellifera*L.) Populations in the United States

We studied the genetic variation of honey bees, *Apis mellifera* L., from central and southern United States by sequencing a portion of the mtDNACO1 and COII gene regions from feral (unmanaged) colonies, swarms and from queen breeder colonies. A total of 27 haplotypes were observed that represent three *A. mellifera* lineages known to have been imported into the U.S. Those lineages include: Eastern European (C, 14 haplotypes); Western European (M, 7 haplotypes); and Middle Eastern (O, 6 haplotypes). Of the samples from 14 queen breeders, we found seven haplotypes (C1, C2, C11, C12, C19, C27, and C31). The most common haplotypes were C1, C2 and C11, which accounted for 82% of the observed haplotypes. Samples from feral colonies and swarms from 11 states, revealed 27 haplotypes. Of the 14 haplotypes observed in the 'C' lineage, seven (C26, C28, C29, C30, C33, C34, C35) were not found in the queen breeder colonies. We found representatives of the Middle Eastern 'O' lineage which has not been imported into the U.S. since the 1880's and is not commercially available. This is the first study to have observed the 'O' lineage in the U.S. This is also the first study to use mtDNA COI-COII sequences extensively on feral colonies, swarms and queen breeder colonies in the U.S.

Journal of Apicultural Research 53(2): 183-187 (2014)

GUEST EDITORIAL: Honey bee genotypes and the environment

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**COMMENT: This paper has no abstract. The following is the concluding paragraph.**

"There is now growing evidence of the adverse effects of the global trade in honey bees, which has led to the spread of novel pests and diseases such as the varroa mite and *Nosema ceranae* (Paxton, 2010; Mutinelli 2011, Fürst et al., 2014). We hope that the evidence provided within the papers of this Special Issue will inspire beekeepers and scientists to explore and appreciate the value of locally bred bees, by developing and supporting breeding programmes. Damage from importations may arise from accompanying pests and pathogens, but it is also inevitable that introduced bees represent a burden to the genetic integrity of local populations. The spread of imported genes into the local population is likely, and the resulting increase in genetic diversity is not universally beneficial. Since maladapted genes will be selected against, this process may well in the short term contribute to colony losses, and is in the long term, unsustainable."

Journal of Apicultural Research 53(2): 205-214 (2014) © IBRA 2014

DOI 10.3896/IBRA.1.53.2.03

The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe

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Received 24 July 2013, accepted subject to revision 3 December 2013, accepted for publication 24 February 2014.

The survival and performance of 597 honey bee colonies, representing five subspecies and 16 different genotypes, were comparatively studied in 20 apiaries across Europe. Started in October 2009, 15.7% of the colonies survived without any therapeutic treatment against diseases until spring 2012. The survival duration was strongly affected by environmental factors (apiary effects) and, to a lesser degree, by the genotypes and

origin of queens. Varroa was identified as a main cause of losses (38.4%), followed by queen problems (16.9%) and Nosema infection (7.3%). On average, colonies with queens from local origin survived 83 days longer compared to non-local origins ( $p < 0.001$ ). This result demonstrates strong genotype by environment interactions. Consequently, the conservation of bee diversity and the support of local breeding activities must be prioritised in order to prevent colony losses, to optimize a sustainable productivity and to enable a continuous adaptation to environmental changes.

**COMMENT: The following short communication from the COLOSS project has no abstract. It has a similar authorship to the one immediately preceding. For brevity, only part of the bibliographic details are quoted.**

Effect of genotype and environment on parasite and pathogen levels in one apiary - a case study  
Francis et al. (2014) Journal of Apicultural Research 53(2): 230-232.

**"our results suggest that bees of local origin are better in tune with environmental factors related to flowering patterns, climatic variation and locally prevailing apicultural methods and therefore may command more sufficient resources to fend off pathogens."**

Open access: <http://www.tandfonline.com/doi/pdf/10.3896/IBRA.1.53.2.14>

Journal of Apicultural Research 53(2): 248-260 (2014) © IBRA 2014

DOI 10.3896/IBRA.1.53.2.06

Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment

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Received 31 January 2014, accepted subject to revision 24 March 2014, accepted for publication 22 April 2014.

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Summary

Honey bee colonies exhibit a wide range of variation in their behaviour, depending on their genetic origin and environmental factors. The COLOSS Genotype-Environment Interactions Experiment gave us the opportunity to investigate the phenotypic expression of the swarming, defensive and hygienic behaviour of 16 genotypes from five different honey bee subspecies in various environmental conditions. In 2010 and 2011, a total of 621 colonies were monitored and tested according to a standard protocol for estimation of expression of these three behavioural traits. The factors: year, genotype, location, origin (local vs. non-local) and season (only for hygienic behaviour) were considered in statistical analyses to estimate their effect on expression of these behaviours. The general outcome of our study is that genotype and location have a significant effect on the analysed traits. For all characters, the variability among locations was higher than the variability among genotypes. We also detected significant variability between the genotypes from different subspecies, generally confirming their known characteristics, although great variability within subspecies was noticed. Defensive and swarming behaviour were each positively correlated across the two years, confirming genetic control of these characters. Defensive behaviour was lower in colonies of local origin, and was negatively correlated with hygienic behaviour. Hygienic behaviour was strongly influenced by the season in which the test was performed. The results from our study demonstrate that there is great behavioural variation among different subspecies and strains. Sustainable protection of local genotypes can be promoted by combining conservation efforts with selection and breeding to improve the appreciation by beekeepers of native stock.

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DOI 10.3896/IBRA.1.53.2.05

#### ORIGINAL RESEARCH ARTICLE

Population dynamics of European honey bee genotypes under different environmental conditions

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Received 8 January 2014, accepted subject to revision 7 March 2014, accepted for publication 28 March 2014.



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#### Summary

Adaptation of honey bees to their environment is expressed by the annual development pattern of the colony, the balance with food sources and the host - parasite balance, all of which interact among each other with changes in the environment. In the present study, we analyse the development patterns over a period of two years in colonies belonging to 16 different genotypes and placed in areas grouped within six environmental clusters across Europe. The colonies were maintained with no chemical treatment against varroa mites. The aim of the study was to investigate the presence of genotype - environment interactions and their effects on colony development, which we use in this study as a measure of their vitality. We found that colonies placed in Southern Europe tend to have lower adult bee populations compared to colonies placed in colder conditions, while the brood population tends to be smaller in the North, thus reflecting the shorter longevity of bees in warmer climates and the shorter brood rearing period in the North. We found that both genotype and environment significantly affect colony development, and that specific adaptations exist, especially in terms of adult bee population and overwintering ability.

Management increases genetic diversity of honey bees via admixture

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Molecular Ecology (2012) doi: 10.1111/j.1365-294X.2012.0

#### Abstract

The process of domestication often brings about profound changes in levels of genetic variation in animals and plants. The honey bee, *Apis mellifera*, has been managed by humans for centuries for both honey and wax production and crop pollination. Human management and selective breeding are believed to have caused reductions in genetic diversity in honey bee populations, thereby contributing to the global declines threatening this ecologically and economically important insect. However, previous studies supporting this claim mostly relied on population genetic comparisons of European and African (or Africanized) honey bee races; such conclusions require reassessment given recent evidence demonstrating that the honey bee originated in Africa and colonized Europe via two independent expansions. We sampled honey bee workers from two managed populations in North America and Europe as well as several old-world progenitor populations in Africa, East and West Europe. Managed bees had highly introgressed genomes representing admixture between East and West European progenitor populations. We found that managed honey bees actually have higher levels of genetic diversity compared with their progenitors in East and West Europe, providing an unusual example whereby human management increases genetic diversity by promoting admixture. The relationship between genetic diversity and honey bee declines is tenuous given that managed bees have more genetic diversity than their progenitors and many viable domesticated animals.

Keywords: admixture, *Apis mellifera*, breeding, management

Received 23 November 2011; revision received 14 March 2012; accepted 23 March 2012

*Apidologie* (1989) 20, 439-450

Inbreeding effects of queen and workers on colony traits in the honey bee

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(received 24 October 1988, accepted 3 July 1989)

#### Summary

Inbreeding coefficients of queens and workers of 5581 controlled mated colonies were estimated. During a period of over 30 years inbreeding increased by 0.15% in queens and 0.06% in workers per year. The highest inbreeding coefficients were 44% in individual queens and 45% in individual worker groups, respectively. Using partial regression, the importance and consequences of inbreeding on colony traits were ascertained. Inbreeding seems to affect the two castes differently. Colony performance with regard to honey and wax is significantly depressed (-6% and -8%, respectively, per 1 % inbreeding) by the inbreeding of the workers. In our material, inbreeding of queens did not affect colony efficiency except when workers were also inbred. Workers can compensate for inbreeding depression of queens, but the reverse is not true. Inbreeding of workers leads to calmer and less aggressive colony behaviour, whereas inbreeding of queens has the opposite effect. Swarming tendency increased with increased inbreeding of workers. In contrast, queens with moder

ate inbreeding have colonies with the highest swarming tendency. Honey production, calmness during examination and swarming tendency show significant interactions between the inbreeding level of queens and workers.

**COMMENT: The following paper is an example of genetic diversity available through open mating in a drone congregation area.**

Proc Biol Sci. 1998 Oct 22; 265(1409): 2009–2014.

doi: 10.1098/rspb.1998.0533

PMCID: PMC1689479

Relatedness among honeybees (*Apis mellifera*) of a drone congregation

E. Baudry, M. Solignac, L. Garnery, M. Gries, J.-M. Cornuet, and N. Koeniger

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Abstract

The honeybee (*Apis mellifera*) queen mates during nuptial flights, in the so-called drone congregation area where many males from surrounding colonies gather. Using 20 highly polymorphic microsatellite loci, we studied a sample of 142 drones captured in a congregation close to Oberursel (Germany). A parentage test based on lod score showed that this sample contained one group of four brothers, six groups of three brothers, 20 groups of two brothers and 80 singletons. These values are very close to a Poisson distribution. Therefore, colonies were apparently equally represented in the drone congregation, and calculations showed that the congregation comprised males that originated from about 240 different colonies. This figure is surprisingly high. Considering the density of colonies around the congregation area and the average flight range of males, it suggests that most colonies within the recruitment perimeter delegated drones to the congregation with an equal probability, resulting in an almost perfect panmixis. Consequently, the relatedness between a queen and her mates, and hence the inbreeding coefficient of the progeny, should be minimized. The relatedness among the drones mated to the same queen is also very low, maximizing the genetic diversity among the different patrilines of a colony.

Trends in Ecology & Evolution

Volume 34, Issue 9, September 2019, Pages 789-798

The Conservation of Native Honey Bees Is Crucial

Fabrice Requier, Lionel Garnery, Patrick L. Kohl, Henry K. Njovu, Christian W. W. Pirk, Robin M. Crewe, Ingolf Steffan-Dewenter

<https://www.sciencedirect.com/science/article/pii/S016953471930117X>

<https://doi.org/10.1016/j.tree.2019.04.008>

The western honey bee, *Apis mellifera*, is globally the most prominent pollinator used for pollination services in crops and is often known only as a domesticated species managed by beekeepers.

Recent studies suggest that the presence of large numbers of introduced managed colonies can threaten wild bee populations. Therefore, proposals to exclude *A. mellifera* colonies from protected areas have arisen.

The diversity of honey bee subspecies in Europe, Africa, and western Asia as a threatened component of the native fauna is underappreciated.

In Europe, wild honey bee populations are endangered, due to lack of nesting sites and hybridization between and the transfer of pathogens and parasites from managed to wild populations.

Natural areas, especially protected areas, are critical for the conservation of the wild populations of the western honey bee in its native range.

<https://link.springer.com/article/10.1007/s10531-017-1404-y>

Thinking beyond Western commercial honeybee hives: towards improved conservation of honey bee diversity

Denise Margaret S. Matias, Christian Borgemeister, Henrik von Wehrden

Biodiversity and Conservation, pp. 1-6, First Online: 15 July 2017 Abstract A decline of wild pollinators, along with a decline of bee diversity, has been a cause of concern among academics and governmental organizations. According to IPBES, a lack of wild pollinator data contributes to difficulties in comprehensively analyzing the regional status of wild pollinators in Africa, Latin America, Asia and

Oceania. It may have also contributed to the prevailing lack of awareness of the diversity of honey bees, of which the managed *Apis mellifera* is often considered as "the (only) honey bee," despite the fact that there are eight other honey bee species extant in Asia. A survey of 100 journal articles published in 2016 shows that 57% of the studies still identified *A. mellifera* as "the honey bee." In total, 80% of studies were conducted solely on *A. mellifera*. This focus on *A. mellifera* has also caused the honey standard of Codex Alimentarius and the European Union to be based solely on *A. mellifera*, causing improper evaluation of honeys from other species. We recommend adapting current standards to reflect the diversity of honey bees and in the process correct failures in the honey market and pave the way towards improved protection of honey bee species and their habitats.

## Beneficial bacteria versus pathogens

**COMMENT:** The papers in this category are relevant in the context of using chemicals in hives that may disrupt the normal microbiome of colonies.

REVIEW: The Bee Microbiome: Impact on Bee Health and Model for Evolution and Ecology of Host-Microbe Interactions

Philipp Engel, a Waldan K. Kwong, b, c Quinn McFrederick, d Kirk E. Anderson, e Seth Michael Barribeau, f James Angus Chandler, g \* R. Scott Cornman, h Jacques Dainat, i Joachim R. de Miranda, j Vincent Doublet, k, l Olivier Emery, a Jay D. Evans, m Laurent Farinelli, n Michelle L. Flenniken, o Fredrik Granberg, p Juris A. Grasis, q Laurent Gauthier, a, b Juliette Hayer, r Hauke Koch, c, s Sarah Kocher, t Vincent G. Martinson, u Nancy Moran, c Monica Munoz-Torres, v Irene Newton, w Robert J. Paxton, k, l Eli Powell, c Ben M. Sadd, x Paul Schmid-Hempel, y Regula Schmid-Hempel, y Se Jin Song, z Ryan S. Schwarz, m Dennis vanEngelsdorp, aa Benjamin Dainat ab, ac Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland

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**ABSTRACT** As pollinators, bees are cornerstones for terrestrial ecosystem stability and key components in agricultural productivity. All animals, including bees, are associated with a diverse community of microbes, commonly referred to as the microbiome. The bee microbiome is likely to be a crucial factor affecting host health. However, with the exception of a few pathogens, the impacts of most members of the bee microbiome on host health are poorly understood. Further, the evolutionary and ecological forces that shape and change the microbiome are unclear. Here, we discuss recent progress in our understanding of the bee microbiome, and we present challenges associated with its investigation. We conclude that global coordination of research efforts is needed to fully understand the complex and highly dynamic nature of the interplay between the bee microbiome, its host, and the environment. High-throughput sequencing technologies are ideal for exploring complex biological systems, including host-microbe interactions. To maximize their value and to improve assessment of the factors affecting bee health, sequence data should be archived, curated, and analyzed in ways that promote the synthesis of different studies. To this end, the BeeBiome consortium aims to develop an online database which would provide reference sequences, archive metadata, and host analytical resources. The goal would be to support applied and fundamental research on bees and their associated microbes and to provide a collaborative framework for sharing primary data from different research programs, thus furthering our understanding of the bee microbiome and its impact on pollinator health.

An emerging paradigm of colony health: microbial balance of the honey bee and hive (*Apis mellifera*)

K. E. Anderson, T. H. Sheehan, B. J. Eckholm, B. M. Mott, G. DeGrandi-Hoffman

Insect. Soc. DOI 10.1007/s00040-011-0194-6 , 30 Aug 2011

**Abstract** Across the globe, honey bee populations have been declining at an unprecedented rate. Managed honey bees are highly social, frequent a multitude of environmental niches, and continually share food, conditions that promote the transmission of parasites and pathogens. Additionally, commercial honey bees used in agriculture are stressed by crowding and frequent transport, and exposed to a plethora of agricultural chemicals and their associated byproducts. When considering this problem, the hive of the honey bee may be best characterized as an extended organism that not only houses developing young and nutrient rich food stores, but also serves as a niche for symbiotic microbial communities that aid in nutrition and defend against pathogens. The niche requirements and maintenance of beneficial honey bee symbionts are largely unknown, as are the ways in which such communities contribute to honey bee nutrition, immunity, and overall health. In this review, we argue that the honey bee should be viewed as a model system to examine the effect of microbial communities on host nutrition and pathogen defense. A systems view focused on the interaction of the honey bee with its associated microbial community is needed to understand the growing agricultural challenges faced by this economically important organism. The road to sustainable honey bee pollination may eventually require the detoxification of agricultural systems, and in the short term, the integrated management of honey bee microbial systems.

Budge, G.E., Adams, I., Thwaites, R., Pietravalle, S., Drew, G.C., Hurst, G.D.D., Tomkies, V., Boonham, N., Brown, M., Identifying bacterial predictors of honey bee health, *Journal of Invertebrate Pathology*(2016), doi: <http://dx.doi.org/10.1016/j.jip.2016.11.003>

Non-targeted approaches are useful tools to identify new or emerging issues in bee health. Here, we utilise next generation sequencing to highlight bacteria associated with healthy and unhealthy honey bee colonies, and then use targeted methods to screen a wider pool of colonies with known health status. Our results provide the first evidence that bacteria from the genus *Arsenophonus* are associated with poor health in honey bee colonies. We also discovered *Lactobacillus* and *Leuconostoc* spp. were associated with healthier honey bee colonies. **Our results highlight the importance of understanding how the wider microbial**

## population relates to honey bee colony health.

MiniReview: Identification and roles of non-pathogenic microflora associated with honey bees

Martha Gilliam

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FEMS Microbiology Letters 155 (1997) 1-10

Received 1 June 1997; accepted 1 July 1997

Abstract

Microorganisms associated with honey bees, *Apis mellifera*, and their food include bacteria (Gram-variable pleomorphic bacteria, *Bacillus* spp., and *Enterobacteriaceae*), molds (primarily aspergilli and penicillia), and yeasts (mainly *Torulopsis* spp.). Eggs, prepupae, pupae, and worker bees emerging from cells as adults are usually free of internal microbes. Microorganisms acquired by larvae through ingestion of contaminated food are usually eliminated through the single defecation that occurs at the end of the feeding period prior to pupation. Emerging adult bees acquire intestinal microflora by food exchange with other bees in the colony and through consumption of pollen. Biochemical contributions of microorganisms to honey bees; the role of microorganisms in the conversion, enhancement, and preservation of pollen stored as bee bread in comb cells; and the production of antimycotic substances by molds and *Bacillus* spp. from honey bee colonies that are resistant to the fungal disease, chalkbrood, are discussed. An association of *Bacillus* spp. with bees including honey bees, stingless bees, and solitary bees from tropical and temperate zones appears to have evolved in which female bees inoculate food sources with these bacteria whose chemical products contribute to the elaboration and/or protection from spoilage of food that is stored in the nest. This association is ancient based on results from stingless bees preserved in amber for 25-40 million years. It is concluded that bees, their products, and their associated microorganisms are potential sources of bioactive products including antimicrobial compounds.

Apidologie DOI: 10.1051/apido/2009065

Novel lactic acid bacteria inhibiting *Paenibacillus* larvae in honey bee larvae

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Forsgren, E., Olofsson, T. C., Vásquez, A. & Fries, I. (2009) Novel lactic acid bacteria inhibiting *Paenibacillus* larvae in honey bee larvae. *Apidologie* online DOI: 10.1051/apido/2009065.

Abstract - We evaluated the antagonistic effects of newly identified lactic acid bacteria (LAB) in the genera *Lactobacillus* and *Bifidobacterium*, originating from the honey stomach, on the honey bee pathogen, *Paenibacillus* larvae. We used inhibition assays on agar plates and honey bee larval bioassays to investigate the effects of honey bee LAB on *P.* larvae growth in vitro and on AFB infection in vivo. The individual LAB phylotypes showed different inhibition properties against *P.* larvae growth on agar plates, whereas a combination of all eleven LAB phylotypes resulted in a total inhibition (no visible growth) of *P.* larvae. Adding the LAB mixture to the larval food significantly reduced the number of AFB infected larvae in exposure bioassays. The results demonstrate that honey bee specific LAB possess beneficial properties for honey bee health. Possible benefits to honey bee health by enhancing growth of LAB or by applying LAB to honey bee colonies should be further investigated.

*Lactobacillus apis* sp. nov., from the stomach of honeybees (*Apis mellifera*), having an in vitro inhibitory effect on the causative agents of American and European foulbrood

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Int. J. Systematic & Evolutionary Biol. (2014), 64,152–157

Abstract

A taxonomic study was performed on Gram-stain-positive, catalase-negative and regular rodshaped bacterial strains R4B<sup>T</sup> and R4C, isolated from the stomachs of honeybees. 16S rRNA gene sequence analysis revealed that the phylogenetic position of the novel strains was within the genus *Lactobacillus*; the highest sequence similarity to R4B<sup>T</sup> was shown by *Lactobacillus acidophilus* BCRC 10695<sup>T</sup> (93.6 %). Lower sequence similarities were found to other obligately homofermentative lactobacilli. A PCR–DGGE method could detect the sequence of the 16S rRNA gene of strain R4B<sup>T</sup> at different developmental stages of honeybees occurring in two different locations in the Czech Republic. The distinctiveness of the strains from other lactobacilli was also confirmed by analysis of sequences of other phylogenetic markers applicable to the taxonomy of the genus *Lactobacillus*, ribotyping and rep-PCR analysis. The DNA G+C content of strain R4B<sup>T</sup> was 41.3 mol%. The predominant cellular fatty acids of strain R4B<sup>T</sup> were C18 : 1 $\omega$ 9c, summed C19 : 1 $\omega$ 6c/C19 : 0cyclo $\omega$ 10c, C16 : 0, summed C18 : 1 $\omega$ 7c/C18 : 1 $\omega$ v6c and summed C16 : 1 $\omega$ 7c/C16 : 1 $\omega$ 6c. The major polar lipids of strain R4B<sup>T</sup> were glycolipids, lipids and phospholipids. Phenotypic and phylogenetic characteristics also confirmed the independent status of the strains at the species level. Interestingly, strain R4B<sup>T</sup> was able to inhibit growth in vitro of *Paenibacillus larvaesubsp.larvae* (causal agent of American foulbrood in honeybees) and *Melissococcus plutonius* (causal agent of European foulbrood). The name *Lactobacillus apissp. nov.* is proposed for this novel taxon; the type strain is R4B<sup>T</sup> (5CCM 8403<sup>T</sup>5LMG 26964<sup>T</sup>).

COMMENT: Although the following paper is on *Apis cerana*, the paper after it contains similar findings in *A. mellifera*.

Journal of Insect Science: Vol. 14 | Article 129

Inhibitory effect of gut bacteria from the Japanese honey bee, *Apis cerana japonica*, against *Melissococcus plutonius*, the causal agent of European foulbrood disease

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Abstract

European foulbrood is a contagious bacterial disease of honey bee larvae. Studies have shown that the intestinal bacteria of insects, including honey bees, act as probiotic organisms. Microbial flora from the gut of the Japanese honey bee, *Apis cerana japonica* F. (Hymenoptera: Apidae), were characterized and evaluated for their potential to inhibit the growth of *Melissococcus plutonius* corrig. (ex White) Bailey and Collins (Lactobacillales: Enterococcaceae), the causative agent of European foulbrood. Analysis of 16S rRNA gene sequences from 17 bacterial strains isolated by using a culture-dependent method revealed that most isolates belonged to *Bacillus*, *Staphylococcus*, and *Pantoea*. The isolates were screened against the pathogenic bacterium *M. plutonius*

by using an in vitro growth inhibition assay, and one isolate (Acja3) belonging to the genus *Bacillus* exhibited inhibitory activity against *M. plutonius*. In addition, in vivo feeding assays revealed that isolate Acja3 decreased the mortality of honey bee larvae infected with *M. plutonius*, suggesting that this bacterial strain could potentially be used as a probiotic agent against European foulbrood.

#### Symbionts as Major Modulators of Insect Health: Lactic Acid Bacteria and Honeybees

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#### Abstract

Lactic acid bacteria (LAB) are well recognized beneficial host-associated members of the microbiota of humans and animals. Yet LAB-associations of invertebrates have been poorly characterized and their functions remain obscure. Here we show that honeybees possess an abundant, diverse and ancient LAB microbiota in their honey crop with beneficial effects for bee health, defending them against microbial threats. Our studies of LAB in all extant honeybee species plus related apid bees reveal one of the largest collections of novel species from the genera *Lactobacillus* and *Bifidobacterium* ever discovered within a single insect and suggest a long (.80 mya) history of association. Bee associated microbiotas highlight *Lactobacillus kunkeei* as the dominant LAB member. Those showing potent antimicrobial properties are acquired by callow honey bee workers from nestmates and maintained within the crop in biofilms, though beekeeping management practices can negatively impact this microbiota. Prophylactic practices that enhance LAB, or supplementary feeding of LAB, may serve in integrated approaches to sustainable pollinator service provision. We anticipate this microbiota will become central to studies on honeybee health, including colony collapse disorder, and act as an exemplar case of insect-microbe symbiosis.

Citation: Va'squez A, Forsgren E, Fries I, Paxton RJ, Flaberg E, et al. (2012) Symbionts as Major Modulators of Insect Health: Lactic Acid Bacteria and Honeybees.

PLoS ONE 7(3): e33188. doi:10.1371/journal.pone.0033188

#### Routes of Acquisition of the Gut Microbiota of the Honey Bee *Apis mellifera*

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#### ABSTRACT

Studies of newly emerged *Apis mellifera* worker bees have demonstrated that their guts are colonized by a consistent core microbiota within several days of eclosion. We conducted experiments aimed at illuminating the transmission routes and spatiotemporal colonization dynamics of this microbiota. Experimental groups of newly emerged workers were maintained in cup cages and exposed to different potential transmission sources. Colonization patterns were evaluated using quantitative real-time PCR (qPCR) to assess community sizes and using deep sequencing of 16S rRNA gene amplicons to assess community composition. In addition, we monitored the establishment of the ileum and rectum communities within workers sampled over time from natural hive conditions. The study verified that workers initially lack gut bacteria and gain large characteristic communities in the ileum and rectum within 4 to 6 days within hives. Typical communities, resembling those of workers within hives, were established in the presence of nurse workers or nurse worker fecal material, and atypical communities of noncore or highly skewed compositions were established when workers were exposed only to oral trophallaxis or hive components (comb, honey, bee bread). The core species of Gram-negative bacteria, *Snodgrassella alvi*, *Gilliamella apicola*, and *Frischella perrara*, were dependent on the presence of nurses or hindgut material, whereas some Gram-positive species were more often transferred through exposure to hive components. These results indicate aspects of the colony life cycle and behavior that are key to the propagation of the characteristic honey bee gut microbiota.

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AEM.01861-14>.

## The Bacterial Communities Associated with Honey Bee (*Apis mellifera*) Foragers

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Tucson, Arizona, United States of America

### Abstract

The honey bee is a key pollinator species in decline worldwide. As part of a commercial operation, bee colonies are exposed to a variety of agricultural ecosystems throughout the year and a multitude of environmental variables that may affect the microbial balance of individuals and the hive. While many recent studies support the idea of a core microbiota in guts of younger in-hive bees, it is unknown whether this core is present in forager bees or the pollen they carry back to the hive. Additionally, several studies hypothesize that the foregut (crop), a key interface between the pollination environment and hive food stores, contains a set of 13 lactic acid bacteria (LAB) that inoculate collected pollen and act in synergy to preserve pollen stores. Here, we used a combination of 454 based 16S rRNA gene sequencing of the microbial communities of forager guts, crops, and corbicular pollen and crop plate counts to show that (1) despite a very different diet, forager guts contain a core microbiota similar to that found in younger bees, (2) corbicular pollen contains a diverse community dominated by hive-specific, environmental or phyllosphere bacteria that are not prevalent in the gut or crop, and (3) the 13 LAB found in culture-based studies are not specific to the crop but are a small subset of midgut or hindgut specific bacteria identified in many recent 454 amplicon-based studies. The crop is dominated by *Lactobacillus kunkeei*, and Alpha 2.2 (*Acetobacteraceae*), highly osmotolerant and acid resistant bacteria found in stored pollen and honey. Crop taxa at low abundance include core hindgut bacteria in transit to their primary niche, and potential pathogens or food spoilage organisms seemingly vectored from the pollination environment. We conclude that the crop microbial environment is influenced by worker task, and may function in both decontamination and inoculation. Citation: Corby-Harris V, Maes P, Anderson KE (2014) The Bacterial Communities Associated with Honey Bee (*Apis mellifera*) Foragers. PLoS ONE 9(4): e95056. doi:10.1371/journal.pone.0095056

### Apidologie

Volume 42, Number 6, 2011 696-699, DOI: 10.1007/s13592-011-0064-2

A scientific note on the lactic acid bacterial flora within the honeybee subspecies *Apis mellifera* (Buckfast), *A. m. scutellata*, *A. m. mellifera*, and *A. m. monticola*

Tobias C. Olofsson, Alejandra Vásquez, Diana Sammataro and Joseph Macharia

No Abstract

### Functional diversity within the simple gut microbiota of the honey bee

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Edited by Gene E. Robinson, University of Illinois at Urbana-Champaign, Urbana, IL, and approved May 21, 2012 (received for review February 27, 2012)

PNAS vol. 109 no. 27, 11002–11007, doi: 10.1073/pnas.1202970109

### Abstract

Animals living in social communities typically harbor a characteristic gut microbiota important for nutrition and pathogen defense. Accordingly, in the gut of the honey bee, *Apis mellifera*, a distinctive microbial community, composed of a taxonomically restricted set of species specific to social bees, has been identified. Despite the ecological and economical importance of honey bees and the increasing concern about population declines, the role of their gut symbionts for colony health and nutrition is unknown. Here,



we sequenced the metagenome of the gut microbiota of honey bees. Unexpectedly, we found a remarkable degree of genetic diversity within the few bacterial species colonizing the bee gut. Comparative analysis of gene contents suggests that different species harbor distinct functional capabilities linked to host interaction, biofilm formation, and carbohydrate breakdown. Whereas the former two functions could be critical for pathogen defense and immunity, the latter one might assist nutrient utilization. In  $\alpha$ -proteobacterial species, we identified genes encoding pectin-degrading enzymes likely involved in the breakdown of pollen walls. Experimental investigation showed that this activity is restricted to a subset of strains of this species providing evidence for niche specialization. Long-standing association of these gut symbionts with their hosts, favored by the eusocial lifestyle of honey bees, might have promoted the genetic and functional diversification of these bee-specific bacteria. Besides revealing insights into mutualistic functions governed by the microbiota of this important pollinator, our findings indicate that the honey bee can serve as a model for understanding more complex gut-associated microbial communities.

The lactic acid bacteria involved in the production of bee pollen and bee bread.

Journal of Apicultural Research Vol. 48 (3) pp. 189 - 195

DOI 10.3896/IBRA.1.48.3.07

Date July 2009

The lactic acid bacteria involved in the production of bee pollen and bee bread.

Author(s) Alejandra Vásquez and Tobias C. Olofsson

Abstract Recently a large flora of lactic acid bacteria (LAB) was identified in the honey stomach of the honey bee *Apis mellifera*. In this study, the presence of this flora in bee pollen and bee bread was investigated. Pollen was collected from the legs of honey bees, and both two week old and two month old bee bread were also obtained for the study. Bacterial isolates cultivated from these bee products were identified using 16S rRNA gene analyzes. The majority of the honey stomach LAB flora was recovered in a viable state from both the pollen and the two week old bee bread, but not from the two month old bee bread. It is demonstrated for the first time that bee bread is probably fermented by the honey stomach LAB flora that has been added to the pollen via regurgitated nectar from the honey stomach. This discovery helps to explain how honey bees standardize the production of bee bread and how it is stored. The presence of the honey stomach LAB and its antimicrobial substances in bee bread also suggests a possible role in the defence against honey bee diseases since the bee bread is consumed by both the larvae and the adult bees.

COMMENT: Slide 8 of the following presentation shows the inhibitory effect of feeding sucrose syrup on lactic acid bacteria counts. Slide 7 appears to indicate that *Paenibacillus larvae*, the primary pathogen in American foulbrood, is ubiquitous in bee colonies. However, other surveys show detectable viable spores in only 8.5-10% of colonies. False negatives can arise by sampling too few bees.

Vásquez, A. & Olofsson, T. (2009) Lactic acid bacteria: can honey bees survive without them? Proceedings of Apimondia 2009. <http://www.apimondia.org/2009/proceedings.htm>

NO ABSTRACT. PDF of slides available:

[http://www.apimondia.com/congresses/2009/Bee-Health/Plenary-](http://www.apimondia.com/congresses/2009/Bee-Health/Plenary-Session/Lactic%20acid%20bacteria,%20can%20honey%20bees%20survive%20without%20them%20-%20OLOFSSON%20Tobias%20C.pdf)

[Session/Lactic%20acid%20bacteria,%20can%20honey%20bees%20survive%20without%20them%20-%20OLOFSSON%20Tobias%20C.pdf](http://www.apimondia.com/congresses/2009/Bee-Health/Plenary-Session/Lactic%20acid%20bacteria,%20can%20honey%20bees%20survive%20without%20them%20-%20OLOFSSON%20Tobias%20C.pdf)

A simple and distinctive microbiota associated with honey bees and bumble bees

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## Abstract

Specialized relationships with bacteria often allow animals to exploit a new diet by providing a novel set of metabolic capabilities. Bees are a monophyletic group of Hymenoptera that transitioned to a completely herbivorous diet from the carnivorous diet of their wasp ancestors. Recent culture-independent studies suggest that a set of distinctive bacterial species inhabits the gut of the honey bee, *Apis mellifera*. Here we survey the gut microbiota of diverse bee and wasp species to test whether acquisition of these bacteria was associated with the transition to herbivory in bees generally. We found that most bee species lack phylotypes that are the same or similar to those typical of *A. mellifera*, rejecting the hypothesis that this dietary transition was symbiont-dependent. The most common bacteria in solitary bee species are a widespread phylotype of *Burkholderia* and the pervasive insect associate, *Wolbachia*. In contrast, several social representatives of corbiculate bees do possess distinctive bacterial phylotypes. Samples of *A. mellifera* harboured the same microbiota as in previous surveys, and closely related bacterial phylotypes were identified in two Asian honey bees (*Apis andreniformis* and *Apis dorsata*) and several bumble bee (*Bombus*) species. Potentially, the sociality of *Apis* and *Bombus* species facilitates symbiont transmission and thus is key to the maintenance of a more consistent gut microbiota. Phylogenetic analyses provide a more refined taxonomic placement of the *A. mellifera* symbionts.

Keywords: *Apis mellifera*, bacterial microbiota, insect symbiosis, microbiology

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Molecular Ecology (2011) 20, 619–628 doi: 10.1111/j.1365-294X.2010.04959.x

I. Janashia, Y. Choiset, H. Rabesona, N. Hwanhlem, N. Bakuradze, N.

Chanishvili, T. Haertlé,

Protection of honeybee *Apis mellifera* by its endogenous and exogenous lactic flora against bacterial infections,

Annals of Agrarian Sciences(2016), doi: 10.1016/j.aasci.2016.07.002.

Lactic acid bacteria (LAB) symbionts of honeybees are certainly playing key roles in their host's colony functioning. The defense against bacterial pathogen invasion by endogenous LAB has been considered as promising and usable phenomenon. This study addresses bacteriocinogeny as one of antibacterial action mode displayed by bacteria. The honeybee endogenous LAB isolated from worker honeybee intestines (61 strains), queen honeybee intestines (16 strains) and beebread(25 strains) were tested for bacteriocin production ability. We checked also well characterized bacteriocin producing LAB strains against bacteria causing American foulbrood (AFB) - *Paenibacillus larvae* aiming possible use of exogenous LAB for control AFB in honeybees and in the same time to observe the vulnerability of endogenous bacteria exposed to bacteriocin producers. We demonstrated that none of 102 studied LAB strains, isolated from worker honeybee intestines, from queen honeybee intestines and from beebread, produced bacteriocins detectable by the well diffusion method (WDM). All of them failed to inhibit two strains of *P. larvae*. Three exogenous bacteriocin-producing LAB strains were tested against the same pathogens and against 25 endogenous bacterial isolates representing 11 different LAB species. The screening showed that all the tested exogenous bacteriocin-producing strains inhibited the tested *P. larvae* strains. The endogenous LAB strains exhibited varied sensitivity profiles when treated with bacteriocin-producing strains. This raises similar challenges to those observed in applications leading to dysbacteriosis, even though the efficacy of these bacteriocins against *P. larvae* in an in vitro system is evident.

Honey bee gut dysbiosis: a novel context of disease ecology

Kirk E Anderson(1,2) and Vincent A Ricigliano(1)

Current Opinion in Insect Science 2017, 22: 1-8

<http://www.sciencedirect.com/science/article/pii/S2214574517300421>

Ecological succession of gut bacteria occurs throughout the life of the adult

Dysbiosis can involve untimely shifts or complete displacement of core bacteria.

Hive bacteria *L. kunkeei* and *P. apium* can completely displace the core gut bacteria.

Early host senescence is a predicted consequence of early gut dysbiosis.

Oxygen dynamics in the gut may play a major role in determining community structure.

Microbial Ecology

<https://doi.org/10.1007/s00248-018-1287-9>

## Gut and Whole-Body Microbiota of the Honey Bee Separate Thriving and Non-thriving Hives

Céline Ribière, Claire Hegarty, Hannah Stephenson, Padraig Whelan, Paul W. O'Toole

22 Nov 2018

The recent worldwide decline of honey bee colonies is a major ecological problem which also threatens pollinated crop production. Several interacting stressors such as environmental pressures and pathogens are suspected. Recently, the gut microbiota has emerged as a critical factor affecting bee health and fitness. We profiled the bacterial communities associated with the gut and whole body of worker bees to assess whether non-thriving colonies could be separated from thriving hives based on their microbial signature. The microbiota of thriving colonies was characterised by higher diversity and higher relative abundance of bacterial taxa involved in sugar degradation that were previously associated with healthy bees (e.g. *Commensalibacter* sp. and *Bartonella apis*). In contrast, the microbiota of non-thriving bees was depleted in health-associated species (e.g. *Lactobacillus apis*), and bacterial taxa associated with disease states (e.g. *Gilliamella apicola*) and pollen degradation (e.g. *G. apicola* and *Bifidobacterium asteroides*) were present in higher abundance compared to thriving colonies. Gut and whole-body microbiota shared a similar dominant core but their comparison showed differences in composition and relative abundance. More differences in taxon relative abundance between gut and whole body were observed in non-thriving bees, suggesting that microbiota associated with other bee organs might also be different. Thus, microbiota profiling could be used as a diagnostic tool in beekeeping practices to predict hive health and guide hive management.

Julia C Jones, Carmelo Fruciano, Falk Hildebrand, Hasan Al Toufalilia, Nicholas J Balfour, Peer Bork, Philipp Engel, Francis LW Ratnieks, William OH Hughes

Gut microbiota composition is associated with environmental landscape in honey bees.

Ecol Evol. 2018;8: 441-451.

<https://doi.org/10.1002/ece3.3597>

There is growing recognition that the gut microbial community regulates a wide variety of important functions in its animal hosts, including host health. However, the complex interactions between gut microbes and environment are still unclear. Honey bees are ecologically and economically important pollinators that host a core gut microbial community that is thought to be constant across populations. Here, we examined whether the composition of the gut microbial community of honey bees is affected by the environmental landscape the bees are exposed to. We placed honey bee colonies reared under identical conditions in two main landscape types for 6 weeks: either oilseed rape farmland or agricultural farmland distant to fields of flowering oilseed rape. The gut bacterial communities of adult bees from the colonies were then characterized and compared based on amplicon sequencing of the 16S rRNA gene. While previous studies have delineated a characteristic core set of bacteria inhabiting the honey bee gut, our results suggest that the broad environment that bees are exposed to has some influence on the relative abundance of some members of that microbial community. This includes known dominant taxa thought to have functions in nutrition and health. Our results provide evidence for an influence of landscape exposure on honey bee microbial community and highlight the potential effect of exposure to different environmental parameters, such as forage type and neonicotinoid pesticides, on key honey bee gut bacteria. This work emphasizes the complexity of the relationship between the host, its gut bacteria, and the environment and identifies target microbial taxa for functional analyses.

## Beekeeper applied antibiotics and antibiotic resistance in gut microbiota

Long-Term Exposure to Antibiotics Has Caused Accumulation of Resistance Determinants in the Gut Microbiota of Honeybees

Baoyu Tian,\* Nibal H. Fadhil, J. Elijah Powell, Waldan K. Kwong, and Nancy A. Moran (2012)

Department of Ecology and Evolutionary Biology, Yale University, West Haven, Connecticut, USAa

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**ABSTRACT** Antibiotic treatment can impact nontarget microbes, enriching the pool of resistance genes available to pathogens and altering community profiles of microbes beneficial to hosts. The gut microbiota of adult honeybees, a distinctive community dominated by eight bacterial species, provides an opportunity to examine evolutionary responses to long-term treatment with a single antibiotic. For decades, American beekeepers have routinely treated colonies with oxytetracycline for control of larval pathogens. Using a

functional metagenomic screen of bacteria from Maryland bees, we detected a high incidence of tetracycline/ oxytetracycline resistance. This resistance is attributable to known resistance loci for which nucleotide sequences and flanking mobility genes were nearly identical to those from human pathogens and from bacteria associated with farm animals. Surveys using diagnostic PCR and sequencing revealed that gut bacteria of honeybees from diverse localities in the United States harbor eight tetracycline resistance loci, including efflux pump genes (*tetB*, *tetC*, *tetD*, *tetH*, *tetL*, and *tetY*) and ribosome protection genes (*tetM* and *tetW*), often at high frequencies. Isolates of gut bacteria from Connecticut bees display high levels of tetracycline resistance. Resistance genes were ubiquitous in American samples, though rare in colonies unexposed for 25 years. In contrast, only three resistance loci, at low frequencies, occurred in samples from countries not using antibiotics in beekeeping and samples from wild bumblebees. Thus, long-term antibiotic treatment has caused the bee gut microbiota to accumulate resistance genes, drawn from a widespread pool of highly mobile loci characterized from pathogens and agricultural sites.

**IMPORTANCE** We found that 50 years of using antibiotics in beekeeping in the United States has resulted in extensive tetracycline resistance in the gut microbiota. These bacteria, which form a distinctive community present in healthy honeybees worldwide, may function in protecting bees from disease and in providing nutrition. In countries that do not use antibiotics in beekeeping, bee gut bacteria contained far fewer resistance genes. The tetracycline resistance that we observed in American samples reflects the capture of mobile resistance genes closely related to those known from human pathogens and agricultural sites. Thus, long-term treatment to control a specific pathogen resulted in the accumulation of a stockpile of resistance capabilities in the microbiota of a healthy gut. This stockpile can, in turn, provide a source of resistance genes for pathogens themselves. The use of novel antibiotics in beekeeping may disrupt bee health, adding to the threats faced by these pollinators.

Tian B, Fadhil NH, Powell JE, Kwong WK, and Moran NA. 2012. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. *mBio* 3(6):e00377-12. doi:10.1128/mBio.00377-12

## **Presence of organic acids in the hive atmosphere (cf. Johann Thür's *Nestduftwärmehindung* – retention of nest scent and heat)**

Vásquez, A. & Olofsson, T. (2009) Lactic acid bacteria: can honey bees survive without them? Proceedings of Apimondia 2009. <http://www.apimondia.org/2009/proceedings.htm>

NO ABSTRACT. PDF of slides available:

<http://www.apimondia.com/congresses/2009/Bee-Health/Plenary-Session/Lactic%20acid%20bacteria,%20can%20honey%20bees%20survive%20without%20them%20-%20OLOFSSON%20Tobias%20C.pdf>

**COMMENT:** This finding does not appear to have been followed up by a fuller report in a peer-reviewed publication.

## **Swarm lures and bait hives for swarm management where swarm suppression is not practised**

Title: Evaluation of synthetic Nasonov pheromone and lemon grass (*Cymbopogon citratus*) essential oil as attractants to swarms of Africanized honey bees (*Apis mellifera*).

Personal Authors: Leopoldino, M. N., Freitas, B. M., Sousa, R. M., Paulino, F. D. G.

Author Affiliation: Departamento de Zootecnia, CCA/Universidade Federal do Ceará, Ceará, Brazil.

Editors: No editors

Document Title: *Ciência Animal*

Leopoldino, M. N., Freitas, B. M., Sousa, R. M. & Paulino, F. D. G. (2002) Avaliação do uso do feromônio de Nasonov sintético e óleo essencial de capim santo (*Cymbopogon citratus*) como atrativos para enxames de abelhas africanizadas (*Apis mellifera*). *Ciência Animal. Fortaleza* **12**(1) 19-23.

Abstract:

This work was conducted at Maravilha farm, Pimenteiras county, State of Piauí, Brazil, from 1 April to 20 June 2000. Its purpose was to evaluate the use of the essential oil lemon grass (*C. citratus*) and a synthetic Nasonov pheromone as attractants to Africanized honey bee (*Apis mellifera*) swarms. The experiment was divided into two phases: firstly, bait hives prepared with strips of beeswax alone were compared to bait hives that in addition to the strips of beeswax were also sprayed on the inside with a 10% solution of lemon grass essential oil and hexane. Secondly, bait hives with beeswax strips + 0.5 ml of lemon grass essential oil were compared to bait hives with beeswax strips + 0.5 ml of synthetic Nasonov pheromone. All treatments were repeated 30 times. Results showed significant differences between treatments in both experimental phases. Bait hives sprayed with lemon grass essential oil attracted 2.5 more swarms than bait hives containing beeswax strips alone ( $\chi^2=15.42$ ;  $P<0.01$ ), and bait hives with synthetic Nasonov pheromone attracted 58% more swarms than bait hives with lemon grass essential oil ( $\chi^2=8.52$ ;  $P<0.01$ ), during the same time interval. It is concluded that despite the fact that all three substances tested attracted swarms, the synthetic Nasonov pheromone was the most efficient and its use by beekeepers should be recommended. Nevertheless, lemon grass essential oil showed itself to be a good alternative if cost or access to synthetic Nasonov pheromone prohibit its use by beekeepers.

Publisher: Universidade Estadual do Ceará, Faculdade de Veterinaria

<http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=20043031043>

#### ORIGINAL RESEARCH ARTICLE

Attraction and direct establishment of primary and secondary honey bee swarms using swarm-tissue sachets  
Alexandros Papachristoforou 1\* and Konstantinos Ilanidis 1

1 Laboratory of Animal Physiology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

Received 19 September 2011, accepted subject to revision 2 May 2012, accepted for publication 19 December 2012.

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#### Summary

In an effort to attract swarms within an apiary and to induce their direct establishment in hive-traps, we investigated the use of a new honey bee swarm attractant in the form of a small sachet containing a wet-wipe tissue, including geranic acid, citral, geraniol and rose oil as attractants. When swarm-tissues sachets were placed in hive traps at a distance of either 5 or 30m from the colonies, 60% of the swarms were established directly and permanently inside hive traps. All established swarms were secondary, headed by virgin queen(s). No primary swarm was attracted. When swarm-tissue sachets were placed in hive traps and were also hung in nearby tree branches, 90.9% of the swarms were attracted. Nine primary swarms formed clusters around the swarm attractant sachets while one secondary swarm was attracted and established directly in a hive trap.

Journal of Apicultural Research 52(2): 8 –11 (2013) DOI 10.3896/IBRA.1.52.2.02

Nest site selection by the honey bee, *Apis mellifera*

Insectes Sociaux

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Pages 323-337

Subject Collection Biomedical and Life Sciences

SpringerLink Date Monday, October 10, 2005

Thomas D. Seeley<sup>1, 2</sup> and Roger A. Morse<sup>1, 2(1)</sup> Museum of Comparative Zoology Laboratories, Harvard University, 02138 Cambridge, Massachusetts, (U.S.A.)

(2) Department of Entomology, Cornell University, 14853 Ithaca, New York, (U.S.A.)

Received: 22 November 1977 Accepted: 9 March 1978

#### Summary

1. Honey bees exhibit preferences in several nest site properties. The following preferences were identified («>» means «preferred to»): nest height, 5>1 m; entrance area, 12.5>75 cm<sup>2</sup>; entrance position, bottom > top of nest cavity, entrance direction, southward > northward; nest cavity volume, 10<40>100 liters.

2. The data also suggest preferences exist for previously inhabited nest cavities and for nest sites beyond 300 m from the parent colony.
3. Nest sites with high exposure and visibility were occupied more rapidly than sites with low exposure and visibility. However, this difference probably reflects differential ease of nest site discovery rather than a preference for exposed nest sites.
4. No preferences were found in the following variables: entrance shape (slit vs. circle), nest cavity shape (cube vs. tall parallelepiped), cavity draftiness (sound vs. drafty), and cavity dryness (wet vs. dry). Cavity draftiness and dryness are probably important to bees, but because bees can seal and waterproof their nests, they may be less demanding about these two nest site variables than about those they cannot modify.
5. The complex process of nest site selection apparently benefits a honey bee colony in several ways, including facilitation of colony defense and hygiene, simplification of nest construction and microclimate control, and reduction of foraging competition with the parent colony.

Seeley, T. D. & Morse, R. A. (1977) Dispersal behaviour of honey bee swarms. *Psyche* 84(3-4) 199-209.

See also: Seeley, T. D. & Morse, R. A. & Nowogrodzki, R. (1989) Bait hives for honey bees. Information bulletin No. 187. A Cornell Cooperative Extension Publication. Cornell University NY.  
[http://ecommons.cornell.edu/bitstream/1813/2653/2/Bait Hives for Honey Bees.pdf](http://ecommons.cornell.edu/bitstream/1813/2653/2/Bait%20Hives%20for%20Honey%20Bees.pdf)

## **Effect of feeding sugar or not feeding honey; role of self-medication and dietary phytochemicals**

COMMENT: For a 49-page review of nectar chemistry see:

Nicholson, S. W. & Thornburg, R. W. (2007) Nectar chemistry. In: *Nectaries and nectar*. Chapter 5. Springer.

Data from several sources shows that whereas sugar contains only carbohydrate, nectar/honey contains Carbohydrate, pH buffered, Probiotic bacteria, e.g. *Lactobacillus*, Bacteria inhibiting AFB and EFB growth, Glucose oxidase generating hydrogen peroxide (antimicrobial), Polyphenols and flavonoids with antimicrobial activity, Antimicrobial diversity of stored honeys, Minerals (Na, K, Ca), Trace elements, Non-protein amino acids – phagostimulant etc., Enzymes e.g. invertase, Vitamins e.g. ascorbic acid (Vitamin C), Up-regulation of detoxification genes

Possible side effects of sugar supplementary nutrition on honey bee health

Davide Frizzera, Simone Del Fabbro, Giacomo Ortis, Virginia Zanni, Renzo Bortolomeazzi, Francesco Nazzi & Desiderato Annoscia  
*Apidologie* (2020)

<https://link.springer.com/article/10.1007/s13592-020-00745-6>

**ABSTRACT** Food shortage, along with biotic stressors, contributes to winter honey bee colony losses. In autumn, to support honey bee colonies and prepare them for the winter season, beekeepers can supply homemade syrups which could contain compounds with possible negative side effects. In this study, we investigated the toxicity of one of those compounds (e.g., hydroxymethylfurfural, HMF) at doses consistent with literature data both to healthy bees and bees challenged with their most important parasite (i.e., *Varroa destructor*). To strengthen available data on HMF concentration in sugar syrups, we also investigated HMF formation in homemade 2:1 inverted sugar syrup, considering, in particular, the influence of temperature or boiling time on different homemade sugar syrups according to their acidity. Finally, we studied the effects of the acidity of sugar syrups on honeybee survival, and tested whether or not sucrose inversion through acidification is really necessary. We show that doses of HMF similar to those reported as sublethal in the literature appear to be non-toxic even to mite infested bees. However, the amount of HMF that can be found in homemade syrups, which increases with temperature and acidity, can be much higher and can cause significant bee mortality. Moreover, we highlighted the detrimental effect of syrups acidity on honeybee survival, suggesting that the addition of lemon or any other acidifying substance to invert the sucrose could be harmful and not necessary. Our results suggest a responsible approach to homemade colony nutrition.

Papežíková, I., Palíková, M., Syrová, E., Zachová, A., Somerlíková, K., Kováčová, V., & Pecková, L. (2019).

Effect of Feeding Honey Bee (*Apis mellifera* Hymenoptera: Apidae) Colonies With Honey, Sugar Solution, Inverted Sugar, and Wheat Starch Syrup on Nosematosis Prevalence and Intensity.

Journal of Economic Entomology. 27 September 2019, doi:10.1093/jee/toz251

<https://academic.oup.com/jee/advance-article-abstract/doi/10.1093/jee/toz251/5575216>

**ABSTRACT** Here, we present the results of a 2-year field trial aimed at testing the effect of overwintering on different feeds on the course of *Nosema ceranae* infection. In August 2015, four experimental bee colony groups were established. After the last honey harvest, each colony was provided with 20 kg of feed, either honey, sugar (3:2 solution in tap water), inverted syrup made of sucrose, or wheat starch syrup. Samples of live bees were collected from each beehive in August (before feeding), November, and May. The following year, feeding and sampling were performed in the same way. Bees were examined microscopically to estimate the percentage of *Nosema*-infected individuals in the sample and the spore number per bee. Fitness parameters were also measured in all colonies. In all hives, presence of *N. ceranae* was confirmed through polymerase chain reaction. *Nosema apis* was not detected in the apiary. Significant differences in nosematosis prevalence and/or intensity were observed between the experimental groups. For most parameters, best results were recorded in the group fed with honey. Worst fitness and highest nosematosis prevalence and intensity were found in colonies fed with wheat starch syrup.

The effect of carbohydrate sources: Sucrose, invert sugar and components of mānuka honey, on core bacteria in the digestive tract of adult honey bees (*Apis mellifera*)

Michelle A. Taylor, Alastair W. Robertson, Patrick J. Biggs, Kate K. Richards, Daniel F. Jones, Shanthi G. Parkar

PLoS ONE 14(12):e0225845.

<https://doi.org/10.1371/journal.pone.0225845>

**ABSTRACT** Bacteria within the digestive tract of adult honey bees are likely to play a key role in the digestion of sugar-rich foods. However, the influence of diet on honey bee gut bacteria is not well understood. During periods of low floral abundance, beekeepers often supplement the natural sources of carbohydrate that honey bees collect, such as nectar, with various forms of carbohydrates such as sucrose (a disaccharide) and invert sugar (a mixture of the monosaccharides glucose and fructose). We compared the effect of these sugar supplements on the relative abundance of bacteria in the gut of bees by feeding bees from a single colony, two natural diets: mānuka honey, a monofloral honey with known antibacterial properties, and a hive diet; and artificial diets of invert sugar, sucrose solution, and sucrose solutions containing synthesised compounds associated with the antibacterial properties of mānuka honey. 16S ribosomal RNA (rRNA)-based sequencing showed that dietary regimes containing mānuka honey, sucrose and invert sugar did not alter the relative abundance of dominant core bacteria after 6 days of being fed these diets. However, sucrose-rich diets increased the relative abundances of three sub-dominant core bacteria, Rhizobiaceae, Acetobacteraceae, and *Lactobacillus kunkeei*, and decreased the relative abundance of *Frischella perrara*, all which significantly altered the bacterial composition. Acetogenic bacteria from the Rhizobiaceae and Acetobacteraceae families increased two- to five-fold when bees were fed sucrose. These results suggest that sucrose fuels the proliferation of specific low abundance primary sucrose-feeders, which metabolise sugars into monosaccharides, and then to acetate.

Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup

Marsha M. Wheeler & Gene E. Robinson

Scientific Reports 4, Article number: 5726 doi:10.1038/srep05726

Received 02 May 2014 Accepted 26 June 2014 Published 17 July 2014

Severe declines in honey bee populations have made it imperative to understand key factors impacting honey bee health. Of major concern is nutrition, as malnutrition in honey bees is associated with immune system impairment and increased pesticide susceptibility. Beekeepers often feed high fructose corn syrup (HFCS) or sucrose after harvesting honey or during periods of nectar dearth. We report that, relative to honey, chronic feeding of either of these two alternative carbohydrate sources elicited hundreds of differences in gene expression in the fat body, a peripheral nutrient-sensing tissue analogous to vertebrate liver and adipose tissues. These expression differences included genes involved in protein metabolism and oxidation-reduction, including some involved in tyrosine and phenylalanine metabolism. Differences

between HFCS and sucrose diets were much more subtle and included a few genes involved in carbohydrate and lipid metabolism. Our results suggest that bees receive nutritional components from honey that are not provided by alternative food sources widely used in apiculture.

<http://www.nature.com/srep/2014/140717/srep05726/full/srep05726.html>

Diversity of honey stores and their impact on pathogenic bacteria of the honeybee, *Apis mellifera*

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doi: 10.1002/ece3.1252

Abstract

Honeybee colonies offer an excellent environment for microbial pathogen development. The highest virulent, colony killing, bacterial agents are *Paenibacillus larva* causing American foulbrood (AFB), and European foulbrood (EFB) associated bacteria. Besides the innate immune defense, honeybees evolved behavioral defenses to combat infections. Foraging of antimicrobial plant compounds plays a key role for this “social immunity” behavior. Secondary plant metabolites in floral nectar are known for their antimicrobial effects. Yet, these compounds are highly plant specific, and the effects on bee health will depend on the floral origin of the honey produced. As worker bees not only feed themselves, but also the larvae and other colony members, **honey is a prime candidate acting as self-medication agent in honeybee colonies to prevent or decrease infections.** Here, we test eight AFB and EFB bacterial strains and the growth inhibitory activity of three honey types. Using a high-throughput cell growth assay, we show that all honeys have high growth inhibitory activity and the two monofloral honeys appeared to be strain specific. The specificity of the monofloral honeys and the strong antimicrobial potential of the polyfloral honey suggest that the diversity of honeys in the honey stores of a colony may be highly adaptive for its “social immunity” against the highly diverse suite of pathogens encountered in nature. This ecological diversity may therefore operate similar to the well-known effects of host genetic variance in the arms race between host and parasite.

Pathogen-associated self-medication behavior in the honeybee *Apis mellifera*

Bogdan I. Gherman & Andreas Denner & Otilia Bobiş & Daniel S. Dezmirean & Liviu A. Mărghitaș & Helge Schlüns & Robin F. A. Moritz & Silvio Erler

Received: 23 January 2014 / Revised: 23 July 2014 / Accepted: 25 July 2014 / Published online: 9 August 2014

#Springer-Verlag Berlin Heidelberg 2014

Abstract

Honeybees, *Apis mellifera*, have several prophylactic disease defense strategies, including the foraging of anti-biotic, antifungal, and antiviral compounds of plant products. Hence, honey and pollen contain many compounds that prevent fungal and bacterial growth and inhibit viral replication. Since these compounds are also fed to the larvae by nurse bees, they play a central role for colony health inside the hive. Here, we show that honeybee nurse bees, infected with the microsporidian gut parasite *Nosema ceranae*, show different preferences for various types of honeys in a simultaneous choice test. Infected workers preferred honeys with a higher antibiotic activity that reduced the microsporidian infection after the consumption of the honey. Since nurse bees feed not only the larvae but also other colony members, this behavior might be a highly adaptive form of therapeutic medication at both the individual and the colony level.

Behav Ecol Sociobiol (2014) 68:1777–1784

DOI 10.1007/s00265-014-1786-8



Pharmacophagy and pharmacophory: mechanisms of self-medication and disease prevention in the honeybee colony (*Apis mellifera*)

Silvio ERLER, Robin F. A. MORITZ

Apidologie Review article

\*INRA, DIB and Springer-Verlag France, 2015

DOI:10.1007/s13592-015-0400-z

Abstract– Apitherapy promises cures for diseases in human folk medicine, but the effects of honeybee produced and foraged compounds on bee health are less known. Yet, hive products should chiefly facilitate medication and sanitation of the honeybees themselves rather than other organisms. We here review the impact of both self-produced gland secretions and foraged hive products (pharmacognosy) on colony health. Although foraged plant-derived compounds vary highly in antibiotic activity depending on the floral and regional origins, secondary plant metabolites in honey, pollen and propolis are important for the antibiotic activity against pathogens and parasites. However, specific bee health-enhancing activities of bee products should clearly be distinguished from the effects of an intact nutrition ensuring the basic immune competence of bees. Further unravelling the interactions among groups of active substances or individual compounds used in concert with specific behavioural adaptations will deepen our understanding of the natural potential of honeybees to maintain colony health.

Apidologie January 2015

Date: 14 Jan 2015

Abscisic acid enhances the immune response in *Apis mellifera* and contributes to the colony fitness

Pedro Negri, Matias D. Maggi, Leonor Ramirez, Leonardo De Feudis, Nicolás Szwariski, Silvina Quintana, Marin J. Eguaras, Lorenzo Lamattina

Abstract

The primary food of adult honey bees (*Apis mellifera*) is honey prepared by bees from nectar, provided by plants in order to stimulate the bee's pollination service. Nectar consists of carbohydrates, amino acids and water, as well as other minor compounds whose proportion varies among plant species and whose biological implications in the honey bee physiology require intense research. Several environmental stressors are causing the decline of bee colonies, and thereby, we tried to connect the nutritional quality of bee's diet with the strength of the bee's immune system. The phytohormone abscisic acid (ABA) is present in nectar, honey and adult honey bees. It has been demonstrated that ABA stimulates innate immune defences in animal cells. However, the influence of ABA on *A. mellifera*'s health and fitness is unknown. Here, we show that honey bees fed with an ABA supplement in field experiments resulted in (i) the appearance of ABA in larvae and adult bees, (ii) enhanced haemocyte response to non-self recognition, (iii) improved wound healing and granulocyte and plasmatocyte activation and (iv) maximum adult bee population after the winter and increased pesticide tolerance. The results indicate that the naturally occurring compound ABA has a positive influence in honey bee immunity. ABA emerges as a potent booster of immune defence in *A. mellifera* and may be useful in addressing the colony losses threatening apiculture and pollination service worldwide.

**COMMENT:** The following paper concerns *Bombus* but is likely to apply to *Apis*.

Secondary metabolites in floral nectar reduce parasite infections in bumblebees

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Regan 2,§ , Winston E. Anthony 2,†† , Jessamyn S. Manson 1,‡‡ and Rebecca E. Irwin 1

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The synthesis of secondary metabolites is a hallmark of plant defence against herbivores. These compounds may be detrimental to consumers, but can also protect herbivores against parasites. Floral nectar commonly contains secondary metabolites, but little is known about the impacts of nectar chemistry on pollinators, including bees. We hypothesized that nectar secondary metabolites could reduce bee parasite infection. We inoculated individual bumblebees with *Crithidia bombi*, an intestinal parasite, and tested effects of eight naturally occurring nectar chemicals on parasite population growth. Secondary metabolites strongly reduced parasite load, with significant effects of alkaloids, terpenoids and iridoid glycosides ranging from 61 to 81%. Using microcolonies, we also investigated costs and benefits of consuming anabasine, the compound with

the strongest effect on parasites, in infected and uninfected bees. Anabasine increased time to egg laying, and Crithidia reduced bee survival. However, anabasine consumption did not mitigate the negative effects of Crithidia, and Crithidia infection did not alter anabasine consumption. Our novel results highlight that although secondary metabolites may not rescue survival in infected bees, they may play a vital role in mediating Crithidia transmission within and between colonies by reducing Crithidia infection intensities.

#### Ecologically Appropriate Xenobiotics Induce Cytochrome P450s in *Apis mellifera*

Reed M. Johnson, Wenfu Mao, Henry S. Pollock, Guodong Niu, Mary A. Schuler, May R. Berenbaum  
PLoS ONE, February 2012, Volume 7, Issue 2, e31051

#### Abstract

**Background:** Honey bees are exposed to phytochemicals through the nectar, pollen and propolis consumed to sustain the colony. They may also encounter mycotoxins produced by *Aspergillus* fungi infesting pollen in beebread. Moreover, bees are exposed to agricultural pesticides, particularly in-hive acaricides used against the parasite *Varroa destructor*. They cope with these and other xenobiotics primarily through enzymatic detoxificative processes, but the regulation of detoxificative enzymes in honey bees remains largely unexplored.

**Methodology/Principal Findings:** We used several approaches to ascertain effects of dietary toxins on bee susceptibility to synthetic and natural xenobiotics, including the acaricide tau-fluvalinate, the agricultural pesticide imidacloprid, and the naturally occurring mycotoxin aflatoxin. We administered potential inducers of cytochrome P450 enzymes, the principal biochemical system for Phase 1 detoxification in insects, to investigate how detoxification is regulated. The drug phenobarbital induces P450s in many insects, yet feeding bees with phenobarbital had no effect on the toxicity of tau-fluvalinate, a pesticide known to be detoxified by bee P450s. Similarly, no P450 induction, as measured by tau-fluvalinate tolerance, occurred in bees fed xanthotoxin, salicylic acid, or indole-3-carbinol, all of which induce P450s in other insects. Only quercetin, a common pollen and honey constituent, reduced tau-fluvalinate toxicity. In microarray comparisons no change in detoxificative gene expression was detected in phenobarbital-treated bees. However, northern blot analyses of guts of bees fed extracts of honey, pollen and propolis showed elevated expression of three CYP6AS P450 genes. Diet did not influence tau-fluvalinate or imidacloprid toxicity in bioassays; however, aflatoxin toxicity was higher in bees consuming sucrose or high-fructose corn syrup than in bees consuming honey.

**Conclusions/Significance:** These results suggest that regulation of honey bee P450s is tuned to chemicals occurring naturally in the hive environment and that, in terms of toxicological capacity, a diet of sugar is not equivalent to a diet of honey.

#### Quotations:

"Midguts of sucrose-fed bees, compared to those consuming honey extract, appeared fragile, flaccid and generally smaller. In order to quantify this apparent difference, a separate bioassay was conducted for the express purpose of quantifying morphological attributes of guts of bees fed different diets. Midguts of bees fed honey extract measured at their broadest point had a statistically greater diameter than did guts of bees fed plain "bee candy" (Figure 2; ANOVA,  $p < 0.01$ ,  $N = 41$ ). Midguts of bees fed unaugmented candy (1.63  $\pm$  0.14 (SD) mm) were smaller in diameter than midguts from bees fed candy with a high dose of honey extract (1.82  $\pm$  0.17 mm; Tukey's HSD,  $p < 0.01$ ), while midguts of bees fed a low dose of honey extract were intermediate in width (1.75  $\pm$  0.15 mm)."

[...]

"Non-honey diets significantly decreased the ability of honey bees to tolerate the natural toxin aflatoxin B1..."

[...]

"When a bee encounters a novel xenobiotic, such as the in-hive acaricides or imidacloprid, with which it has not coevolved, these synthetic compounds may not activate the same molecular pathways as are activated by naturally occurring xenobiotics in hive products."

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0031051>

Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*  
Wenfu Mao<sup>a</sup>, Mary A. Schuler<sup>b</sup>, and May R. Berenbaum<sup>a</sup>,  
<sup>1</sup>Departments of <sup>a</sup> Entomology and <sup>b</sup> Cell and Developmental Biology, University of Illinois at Urbana–Champaign, Urbana, IL 61801

Abstract

As a managed pollinator, the honey bee *Apis mellifera* is critical to the American agricultural enterprise. Recent colony losses have thus raised concerns; possible explanations for bee decline include nutritional deficiencies and exposures to pesticides and pathogens. We determined that constituents found in honey, including p-coumaric acid, pinocembrin, and pinobanksin 5-methyl ether, specifically induce detoxification genes. These inducers are primarily found not in nectar but in pollen in the case of p-coumaric acid (a monomer of sporopollenin, the principal constituent of pollen cell walls) and propolis, a resinous material gathered and processed by bees to line wax cells. RNA-seq analysis (massively parallel RNA sequencing) revealed that p-coumaric acid specifically up-regulates all classes of detoxification genes as well as select antimicrobial peptide genes. This up-regulation has functional significance in that adding p-coumaric acid to a diet of sucrose increases midgut metabolism of coumaphos, a widely used in-hive acaricide, by ~60%. As a major component of pollen grains, p-coumaric acid is ubiquitous in the natural diet of honey bees and may function as a nutraceutical regulating immune and detoxification processes. The widespread apicultural use of honey substitutes, including high-fructose corn syrup, may thus compromise the ability of honey bees to cope with pesticides and pathogens and contribute to colony losses.

<https://academic.oup.com/jee/article-abstract/doi/10.1093/jee/tox193/3988364/Nectar-and-Pollen-Phytochemicals-Stimulate-Honey?redirectedFrom=fulltext>

Nectar and Pollen Phytochemicals Stimulate Honey Bee (Hymenoptera: Apidae)

Immunity to Viral Infection

Journal of Economic Entomology, 2017, 1-14

doi: 10.1093/jee/tox193

Evan C. Palmer-Young, Cansu O. Tozkar, Ryan S. Schwarz, Yanping Chen, Rebecca E. Irwin, Lynn S. Adler, and Jay D. Evans

Parasites and pathogens are implicated in honey bee colony losses, and honey bees may also spread infection to wild pollinators. Bees consume nectar and pollen, which contain phytochemicals that can positively or negatively affect pollinator health. Certain phytochemicals can reduce parasite loads in humans and other animals. Understanding how phytochemicals affect honey bee infection and survival could help identify optimal forage sources and phytochemical treatments to ameliorate disease. We fed honey bees seven dietary phytochemicals to evaluate whether phytochemical consumption would treat preexisting infection in mature bees, or mitigate infection in young bees either inside or outside of their colonies. Phytochemicals were generally well-tolerated at levels documented in nectar, honey, and pollen, although clove oil and thymol increased mortality at high doses. Six of seven tested phytochemicals significantly increased antimicrobial peptide expression by 12.9 to 61-fold in older bees after 7 d consumption. Short-term (<24 h) phytochemical consumption reduced levels of Deformed wing virus (DWV) up to 500-fold in young bees released into field colonies. However, with the exception of high-dose clove oil, our phytochemical treatments did not alter infection with *Lotmaria passim* or *Nosema ceranae*. Phytochemicals also lacked antiviral effects for pollen-deprived bees reared outside the colony. Our results suggest that phytochemicals have potential therapeutic value for honey bees infected with DWV. Short-term phytochemical consumption may be sufficient to confer benefits against infection. Phytochemical concentrations that reduced disease were comparable with naturally occurring floral concentrations, suggesting that flowers could serve as seasonally varied, serially consumed pollinator medicines.

Impacts of Dietary Phytochemicals in the Presence and Absence of Pesticides on Longevity of Honey Bees (*Apis mellifera*)

by Ling-Hsiu Liao, Wen-Yen Wu and May R. Berenbaum \*

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Insects 2017, 8(1), 22; <https://doi.org/10.3390/insects8010022>

<https://www.mdpi.com/2075-4450/8/1/22>

Because certain flavonols and phenolic acids are found in pollen and nectar of most angiosperms, they are routinely ingested by *Apis mellifera*, the western honey bee. The flavonol quercetin and the phenolic acid p-coumaric acid are known to upregulate detoxification enzymes in adult bees; their presence or absence in the

diet may thus affect the toxicity of ingested pesticides. We conducted a series of longevity assays with one-day-old adult workers to test if dietary phytochemicals enhance longevity and pesticide tolerance. One-day-old bees were maintained on sugar syrup with or without casein (a phytochemical-free protein source) in the presence or absence of quercetin and p-coumaric acid as well as in the presence or absence of two pyrethroid insecticides, bifenthrin and  $\beta$ -cyfluthrin. Dietary quercetin (hazard ratio, HR = 0.82), p-coumaric acid (HR = 0.91) and casein (HR = 0.74) were associated with extended lifespan and the two pyrethroid insecticides, 4 ppm bifenthrin (HR = 9.17) and 0.5 ppm  $\beta$ -cyfluthrin (HR = 1.34), reduced lifespan. Dietary quercetin enhanced tolerance of both pyrethroids; p-coumaric acid had a similar effect trend, although of reduced magnitude. Casein in the diet appears to eliminate the life-prolonging effect of p-coumaric acid in the absence of quercetin. Collectively, these assays demonstrate that dietary phytochemicals influence honey bee longevity and pesticide stress; substituting sugar syrups for honey or yeast/soy flour patties may thus have hitherto unrecognized impacts on adult bee health

High Fructose Corn Syrup Down-Regulates the Glycolysis Pathway in *Apis mellifera*

Michelle R. Jennette (Honours degree thesis)

5-9-2017

[http://vc.bridgew.edu/cgi/viewcontent.cgi?article=1217&context=honors\\_proj](http://vc.bridgew.edu/cgi/viewcontent.cgi?article=1217&context=honors_proj)

Over the past few years, honeybee populations (*Apis mellifera*) have drastically decreased due to Colony Collapse Disorder (CCD). CCD occurs when most of the honeybees abandon their hive and queen, and do not return. Around the same time CCD became commonly identified, commercial beekeepers switched the bee's food from sucrose to a less expensive, but more processed substitute of high fructose corn syrup (HFCS), suggesting there might be a relationship between the types of food and CCD. Therefore, the goal of this project is to identify changes in carbohydrate metabolism due to HFCS. To determine these changes, gene expression was measured within the glycolysis and gluconeogenesis pathways. For seven days, bees from 9 hives were fed honey, high fructose corn syrup (HFCS), corn syrup (CS), and sucrose. After termination, mRNA was extracted and cDNA synthesized for Quantitative PCR (qPCR) analysis with SYBR green to detect changes in expression for 14 genes in these pathways. In the glycolysis pathway, the enzymes glucokinase (GK1), phosphofructokinase (PFK), triose phosphate isomerase (TPS1), pyruvate kinase (PK2) and lactate dehydrogenase (LDHA) were down-regulated in HFCS compared to honey. Further, when bees were fed CS, they down-regulated most of the genes that are down-regulated in HFCS. However, these genes were not changed when bees were fed sucrose. For genes in both glycolysis and gluconeogenesis, gene expression did not change when given HFCS. This was also observed for genes in just gluconeogenesis. In summary, HFCS and CS diets both lead to down-regulated genes specific to the glycolysis pathway when compared to honey.

<https://link.springer.com/article/10.1007/s11829-017-9553-1>

Arthropod-Plant Interactions, pp 1-8, 31 July 2017

The immunological dependence of plant-feeding animals on their host's medical properties may explain part of honey bee colony losses

Erik Tihelka

The honey bee (*Apis mellifera*) is an important pollinator of agricultural and horticultural crops, but also of wild flowers. The species has been facing declines in many areas of the world, the causes being identified as multifactorial. Recently, it has been theorised that some plant-dwelling animals may develop a dependence on the medicinal properties of their host's plant's secondary metabolites. Here, the question of honey bee self-medication using organic materials, namely propolis, nectar, honey, honeydew, pollen, wood, and algae for self-medication is addressed. Self-medication in honey bees is a largely unexplored area and thus a comprehensive overview of the field is provided. Prior studies suggest that recent honey bee colony declines are driven by decreased forage plant availability. The problem is expanded and it is suggested, that if honey bees developed a dependence on medical properties of some disappearing plants or materials, this could explain a part of the colony losses observed around the world. To date, convincing evidence points towards self-medication with honey and propolis. Bees also contact plant secondary metabolites, fatty acids, essential oils, and microorganisms that are active against the causative agents of American foulbrood, European foulbrood, nosemosis, chalkbrood, stonebrood, and varroasis. In the future, selected taxa of plants with medicinal properties may be planted to boost honey bee health without chemotherapy. Future directions of research are discussed.

## Comb as a vibration communication medium

COMMENT: In *The Buzz about bees* Jürgen Tautz suggests that comb built on foundation in wooden frames disturbs the vibration communication through the comb. In Fig. 7.25 he shows how the bees remove wax round the edges of the such comb to make the vibration possible again. However, also consider the paper below on plastic comb foundation.

The Journal of Experimental Biology 199, 2585–2594 (1996)

### TRANSMISSION OF VIBRATION ACROSS HONEYCOMBS AND ITS DETECTION BY BEE LEG RECEPTORS

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Accepted 5 August 1996

#### Abstract

Vibration of the rims of open cells in a honeycomb, applied in the plane of the comb face, is transmitted across, the comb. Attenuation or amplification of the vibratory, signal depends on its frequency and on the type of comb. In general, framed combs, both large and small, strongly, attenuate higher frequencies, whereas these are amplified, in small open combs. The very poor transmission, properties of the large framed combs used in commercial, hives may explain the bees' habit of freeing an area of comb, from the frame in those areas used for dancing. Extracellular electrical recordings from the leg of a, honeybee detect large action potentials from receptors that, monitor extension of the tibia on the femur. Measurements, of threshold displacement amplitudes show these receptors, to be sensitive to low frequencies. The amplification, properties of unframed combs extend the range of these, receptor systems to include frequencies that are emitted by, the bee during its dance, namely the 15 Hz abdomen waggle, and 250 Hz thorax vibration.

Key words: honeybee, waggle dance, comb vibration, communication, *Apis mellifera carnica*.

The Journal of Experimental Biology 203, 1573–1579 (2000)

### BEHAVIOUR-LOCKED SIGNAL ANALYSIS REVEALS WEAK 200–300 Hz COMB, VIBRATIONS DURING THE HONEYBEE WAGGLE DANCE, JAMES C. NIEH\* AND JÜRGEN TAUTZ‡

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Accepted 25 February; published on WWW 18 April 2000

#### Abstract

Waggle-dancing honeybees produce vibratory, movements that may facilitate communication by, indicating the location of the waggle dancer. However, an, important component of these vibrations has never been, previously detected in the comb. We developed a method, of fine-scale behavioural analysis that allowed us to analyze, separately comb vibrations near a honeybee waggle dancer, during the waggle and return phases of her dance. We, simultaneously recorded honeybee waggle dances using, digital video and laser-Doppler vibrometry, and performed, a behaviour-locked Fast Fourier Transform analysis on the, substratum vibrations. This analysis revealed significantly, higher-amplitude 200–300 Hz vibrations during the waggle, phase than during the return phase ( $P=0.012$ ). We found, no significant differences in the flanking frequency regions, between 100–200 Hz ( $P=0.227$ ) and 300–400 Hz ( $P=0.065$ ). We recorded peak waggle phase vibrations from 206 to, 292 Hz ( $244\pm 28$  Hz; mean  $\pm$  S.D.,  $N=11$ ). The maximum, measured signal – noise level was +12.4 dB during the, waggle phase (mean  $+5.8\pm 2.7$  dB). The maximum, vibrational velocity, calculated from a filtered signal, was,  $128\mu\text{ms}^{-1}$ , peak-to-peak, corresponding to a displacement, of  $0.09\mu\text{m}$  peak-to-peak at 223 Hz. On average, we, measured a vibrational velocity of  $79\pm 28\mu\text{ms}^{-1}$ , peak-to-peak from

filtered signals. These signal amplitudes overlap, with the detection threshold of the honeybee subgenual, organ.

The Journal of Experimental Biology 204, 3737–3746 (2001)

Phase reversal of vibratory signals in honeycomb may assist dancing honeybees, to attract their audience,

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Accepted 9 August 2001

Abstract

Forager honeybees dancing on the comb are able to, attract dance-followers from distances across the comb, that are too remote for tactile or visual signals to play a, role. An alternative signal could be the vibrations of the, comb at 200–300 Hz generated by dancing bees but which,, without amplification, may not be large enough to alert, remote dance-followers. We describe here, however, an, unexpected property of honeycomb when it is subjected to, vibration at around 200 Hz that would represent an, effective amplification of the vibratory signals for remote, dance-followers. We find that, at a specific distance from, the origin of an imposed vibration, the walls across a, single comb cell abruptly reverse the phase of their, displacement and move in opposite directions to one, another. Behavioural measurements show that the, distance from which the majority of remote dancefollowers are recruited coincides with the location of this, phase-reversal phenomenon relative to the signal source., We propose that effective signal amplification by the, phase-reversal phenomenon occurs when bees straddle a, cell across which the phase reversal is expressed. Such a, bee would be subjected to a situation in which the legs, were moving towards and away from one another instead, of in the same direction. In this manner, remote dancefollowers could be alerted to a dancer performing in their, vicinity.

Apidologie 36 (2005) 513–521

Does plastic comb foundation hinder waggle dance communication?

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Received 8 February 2005 – revised 16 March 2005 – accepted 16 March 2005

Published online 13 September 2005

Abstract – In recent years, plastic comb foundation has become widely used by beekeepers but it has not been studied to see if it hinders recruitment communication by reducing the transmission of the comb vibrations produced by bees performing waggle dances. We used laser vibrometry to compare combs built with beeswax foundation vs. plastic foundation in terms of transmission of dance vibrations. We also used behavioral experiments to compare the recruitment effectiveness of dances performed on combs built with beeswax foundation vs. plastic foundation. We found that combs built with plastic foundation are markedly poorer at transmitting the 250 Hz vibrations produced by dancing bees. Nevertheless, we found no evidence of reduced effectiveness of dances performed on combs built with plastic foundation vs. combs built with beeswax foundation. Evidently, a comb built with plastic foundation provides a fully suitable substrate for waggle dance communication.

## **Beekeeping contributes to rather than detracts from conservation biology**

Estimating the Density of Honeybee Colonies across Their Natural Range to Fill the Gap in Pollinator Decline Censuses

RODOLFO JAFFÉ, VINCENT DIETEMANN, MIKE H. ALLSOPP, CECILIA COSTA, ROBIN M. CREWE, RAFFAELE DALL’OLIO, PILAR DE LA RÚA, MOGBEL A. A. EL-NIWEIRI, INGEMAR FRIES, NIKOLA KEZIC, MICHAEL S. MEUSEL, ROBERT J. PAXTONTAHER SHAIBI, ECKART STOLLE, ROBIN F.A. MORITZ

Abstract: Although pollinator declines are a global biodiversity threat, the demography of the western honeybee (*Apis mellifera*) has not been considered by conservationists because it is biased by the activity of beekeepers. To fill this gap in pollinator decline censuses and to provide a broad picture of the current status of honeybees across their natural range, we used microsatellite genetic markers to estimate colony densities and genetic diversity at different locations in Europe, Africa, and central Asia that had different patterns of land use. Genetic diversity and colony densities were highest in South Africa and lowest in Northern Europe and were correlated with mean annual temperature. Confounding factors not related to climate, however, are also likely to influence genetic diversity and colony densities in honeybee populations. Land use showed a significantly negative influence over genetic diversity and the density of honeybee colonies over all sampling locations. In Europe honeybees sampled in nature reserves had genetic diversity and colony densities similar to those sampled in agricultural landscapes, which suggests that the former are not wild but may have come from managed hives. Other results also support this idea: putative wild bees were rare in our European samples, and the mean estimated density of honeybee colonies on the continent closely resembled the reported mean number of managed hives. Current densities of European honeybee populations are in the same range as those found in the adverse climatic conditions of the Kalahari and Saharan deserts, which suggests that beekeeping activities do not compensate for the loss of wild colonies. **Our findings highlight the importance of reconsidering the conservation status of honeybees in Europe and of regarding beekeeping not only as a profitable business for producing honey, but also as an essential component of biodiversity conservation.**

Conservation Biology, Volume 24, No. 2, 583–593, 2009 DOI: 10.1111/j.1523-1739.2009.01331.x

## **International trade in honey bees spreads pests and pathogens**

The spread of pathogens through trade in honey bees and their products (including queen bees and semen): overview and recent developments

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Summary

International trade in bees and bee products is a complex issue, affected by their different origins and uses. The trade in bees, which poses the main risk for disease dissemination, is very active and not all transactions may be officially registered by the competent authorities. Globally, bee health continues to deteriorate as pathogens, pests, parasites and diseases are spread internationally through legitimate trade, smuggling and well-intentioned but illadvised bee introductions by professionals. International trade rules strengthen the ability of many countries to protect bee health while trading but also carry obligations. Countries that are Members of the World Trade Organization (WTO) should only restrict imports to protect against identifiable health risks. If imports are safe, trade should be permitted. The trading rules of the WTO have given greater importance to the international standards applicable to bee health, developed by the World Organisation for Animal Health, which aims to prevent the spread of animal diseases while facilitating international trade in animals and animal products.

Rev. sci. tech. Off. int. Epiz., 2011, 30(1), 257-271

Quo vadis *Aethina tumida*? Biology and control of small hive beetles

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Received 15 June 2015–Revised 8 November 2015–Accepted 30 December 2015

Abstract– Small hive beetles (SHBs) are generalists native to sub-Saharan Africa and reproduce in association with honeybees, bumblebees, stingless bees, fruits and meat. The SHB has recently become an

invasive species, and introductions have been recorded from America, Australia, Europe and Asia since 1996. While SHBs are usually considered a minor pest in Africa, they can cause significant damage to social bee colonies in their new ranges. Potential reasons for differential impact include differences in bee behaviour, climate and release from natural enemies. Here, we provide an overview on biology, distribution, pest status, diagnosis, control and prevention to foster adequate mitigation and stimulate future research. SHBs have become a global threat to both apiculture and wild bee populations, but our knowledge of this pest is still limited, creating demand for more research in all areas of its biology.

Apidologie (2016) 47:427–466 Review article. This article is published with open access at Springerlink.com DOI:10.1007/s13592-016-0426-x

### Biology and control of Varroa destructor

Peter Rosenkranz, \* , Pia Aumeier b , Bettina Ziegelmann a

Journal of Invertebrate Pathology 103 (2010) S96–S119

The ectoparasitic honey bee mite *Varroa destructor* was originally confined to the Eastern honey bee *Apis cerana*. After a shift to the new host *Apis mellifera* during the first half of the last century, the parasite dispersed world wide and is currently considered the major threat for apiculture. The damage caused by Varroosis is thought to be a crucial driver for the periodical colony losses in Europe and the USA and regular Varroa treatments are essential in these countries. Therefore, Varroa research not only deals with a fascinating host–parasite relationship but also has a responsibility to find sustainable solutions for the beekeeping.

This review provides a survey of the current knowledge in the main fields of Varroa research including the biology of the mite, damage to the host, host tolerance, tolerance breeding and Varroa treatment. We first present a general view on the functional morphology and on the biology of the Varroa mite with special emphasis on host–parasite interactions during reproduction of the female mite. The pathology section describes host damage at the individual and colony level including the problem of transmission of secondary infections by the mite. Knowledge of both the biology and the pathology of Varroa mites is essential for understanding possible tolerance mechanisms in the honey bee host. We comment on the few examples of natural tolerance in *A. mellifera* and evaluate recent approaches to the selection of Varroa tolerant honey bees. Finally, an extensive listing and critical evaluation of chemical and biological methods of Varroa treatments is given.

This compilation of present-day knowledge on Varroa honey bee interactions emphasizes that we are still far from a solution for Varroa infestation and that, therefore, further research on mite biology, tolerance breeding, and Varroa treatment is urgently needed.

## Non-invasive colony monitoring

**COMMENT:** This topic was not included in earlier editions of this compilation, but it is a rapidly growing field due to modern informaiion technology systems.

Honey bee vibration monitoring using the 805M1 accelerometer Martin Bencsik and Michael I Newton

[http://irep.ntu.ac.uk/id/eprint/35332/1/12754\\_Newton.pdf](http://irep.ntu.ac.uk/id/eprint/35332/1/12754_Newton.pdf)

In this work we demonstrate that the 805M1 single axis analogue output accelerometer can be used to monitor honey bee activity and requires only a low cost microcontroller with an audio shield to log the data. We present accelerometer output signals demonstrating the ability to capture individual honey bee ‘whooping’ signals as well as long term amplitude monitoring, indicating the brood cycle, using this affordable measurement system.

### A Low-Cost Multi-Modal Sensor Network

for the Monitoring of

Honeybee Colonies/Hives

Donald HOWARD, Olga DURAN and Gordon HUNTER

<https://eprints.kingston.ac.uk/41328/1/Howard-D-41328-VoR.pdf>

Honeybees, which play an essential role as pollinators, have suffered a significant decline in recent years. Different types of sensors, including acoustic, chemical, vision, mass and temperature, can provide



important information to assess their well-being. However, a multi-modal sensor system would need to be economical and affordable in order to be used on a large scale, including by less wealthy farmers or beekeepers. We present details of a low-cost sensor network system to allow the continuous monitoring of honeybee hives in a non-invasive manner, discussing its advantages relative to other existing systems for the same purpose, and initial results from the deployment of such a system in four hives.

QUOTE FROM PAPER: As described in our previous paper [4], we have adopted the same philosophy as Neumann & Blacquière [1] -- namely allowing the bees to live as naturally as feasible -- and the guidelines of Seeley [2, 3] for hive size, design, location and orientation, subject to a constraint of making the hives accessible for maintenance."

Insects 2018, 9(4), 176; <https://doi.org/10.3390/insects9040176>  
Breakfast Canyon Discovered in Honeybee Hive Weight Curves  
Niels Holst and William G. Meikle

Electronic devices to sense, store, and transmit data are undergoing rapid development, offering an ever-expanding toolbox for inventive minds. In apiculture, both researchers and practitioners have welcomed the opportunity to equip beehives with a variety of sensors to monitor hive weight, temperature, forager traffic and more, resulting in huge amounts of accumulated data. The problem remains how to distil biological meaning out of these data. In this paper, we address the analysis of beehive weight monitored at a 15-min resolution over several months. Inspired by an overlooked, classic study on such weight curves we derive algorithms and statistical procedures to allow biological interpretation of the data. Our primary finding was that an early morning dip in the weight curve ('Breakfast Canyon') could be extracted from the data to provide information on bee colony performance in terms of foraging effort. We include the data sets used in this study, together with R scripts that will allow other researchers to replicate or refine our method.

Close-Range Photogrammetry and Infrared Imaging for Non-Invasive Honeybee Hive Population Assessment ISPRS Int. J. Geo-Inf. 2018, 7, 350; doi:10.3390/ijgi7090350 Luis López-Fernández, Susana Lagüela, Pablo Rodríguez-González, José Antonio Martín-Jiménez and Diego González-Aguilera  
[http://scholar.google.co.uk/scholar\\_url?url=http://www.mdpi.com/2220-9964/7/9/350/pdf&hl=en&sa=X&d=16253100067508650574&scisig=AAGBfm0Ewmkr6JMLe175OwupLwmi-993Mw&noss=1&oi=scholar&hist=WHemHUQAAAAJ:11163700300156213052:AAGBfm2e\\_8dobtSBH3BOjjhJc\\_VRdBi4LQ](http://scholar.google.co.uk/scholar_url?url=http://www.mdpi.com/2220-9964/7/9/350/pdf&hl=en&sa=X&d=16253100067508650574&scisig=AAGBfm0Ewmkr6JMLe175OwupLwmi-993Mw&noss=1&oi=scholar&hist=WHemHUQAAAAJ:11163700300156213052:AAGBfm2e_8dobtSBH3BOjjhJc_VRdBi4LQ)

Close-range photogrammetry and thermographic imaging techniques are used for the acquisition of all the data needed for the non-invasive assessment of a honeybee hive population. Temperature values complemented with precise 3D geometry generated using novel close-range photogrammetric and computer vision algorithms are used for the computation of the inner beehive temperature at each point of its surface. The methodology was validated through its application to three reference beehives with different population levels. The temperatures reached by the exterior surfaces of the hives showed a direct correlation with the population level. In addition, the knowledge of the 3D reality of the hives and the position of each temperature value allowed the positioning of the bee colonies without the need to open the hives. This way, the state of honeybee hives regarding the growth of population can be estimated without disturbing its natural development.

## Health impacts of migratory beekeeping

Home sick: impacts of migratory beekeeping on honey bee (*Apis mellifera*) pests, pathogens, and colony size

Samantha A. Alger, P. Alexander Burnham, Zachary S. Lamas, Alison K. Brody, Leif L. Richardson PeerJ, November 2, 2018 <https://peerj.com/articles/5812/>

Honey bees are important pollinators of agricultural crops and the dramatic losses of honey bee colonies have risen to a level of international concern. Potential contributors to such losses include pesticide exposure, lack of floral resources and parasites and pathogens. The damaging effects of all of these may be exacerbated by apicultural practices. To meet the pollination demand of US crops, bees are transported to

areas of high pollination demand throughout the year. Compared to stationary colonies, risk of parasitism and infectious disease may be greater for migratory bees than those that remain in a single location, although this has not been experimentally established. Here, we conducted a manipulative experiment to test whether viral pathogen and parasite loads increase as a result of colonies being transported for pollination of a major US crop, California almonds. We also tested if they subsequently transmit those diseases to stationary colonies upon return to their home apiaries. Colonies started with equivalent numbers of bees, however migratory colonies returned with fewer bees compared to stationary colonies and this difference remained one month later. Migratory colonies returned with higher black queen cell virus loads than stationary colonies, but loads were similar between groups one month later. Colonies exposed to migratory bees experienced a greater increase of deformed wing virus prevalence and load compared to the isolated group. The three groups had similar infestations of *Varroa* mites upon return of the migratory colonies. However, one month later, mite loads in migratory colonies were significantly lower compared to the other groups, possibly because of lower number of host bees. Our study demonstrates that migratory pollination practices has varying health effects for honey bee colonies. Further research is necessary to clarify how migratory pollination practices influence the disease dynamics of honey bee diseases we describe here.

Long-Distance Transportation Causes Temperature Stress in the Honey Bee, *Apis mellifera* (Hymenoptera: Apidae)

Dacotah Melicher, Elisabeth S. Wilson, Julia H. Bowsher, Steve S. Peterson, George D. Yocum, and Joseph P. Rinehart.

Environmental Entomology, XX(XX), 2019, 1–11

<https://academic.oup.com/ee/advance-article/doi/10.1093/ee/nvz027/5423020>

Pollination services provided by the honey bee, *Apis mellifera* (Hymenoptera: Apidae, Linnaeus, 1758) have broad economic impacts and are necessary for production of a diversity of important crops. Hives may be transported multiple times per year to provide pollination. To test how temperature may contribute to transportation stress, temperature sensors were placed in hives in different locations and orientations on the trailer during shipping. Colony size prior to shipping significantly contributed to loss of population immediately after shipping which contributed to colony failure with smaller colonies more likely to fail and fail faster. Colony size also affects thermoregulation and temperature stress. Internal hive temperature varies significantly based on location and orientation. While colonies near the front and rear of the trailer and those oriented toward the center aisle had significantly different average internal temperatures, colony size best predicts loss of thermoregulation. Additionally, we profiled gene expression at departure, on arrival, and after a recovery period to identify transcriptional responses to transportation. Functional and enrichment analysis identified increased methylation and decreased ribosomal and protein-folding activity. Pheromone and odorant-binding transcripts were up-regulated after transportation. After recovery, transcripts associated with defense response, immune activity, and heat shock decreased, while production of antibiotic peptides increased. We conclude that hives experience considerable temperature stress possibly caused by turbulent airflow in exposed locations. Transportation stress should be considered an important component of annual colony losses which can be mitigated with improved management strategies.

Antimicrobial properties of brood comb (propolis)

Stephanie K. Murray (thesis for a MSc.)

Effects of used brood comb and propolis on honey bees (*Apis mellifera* L.) and their associated bacterium, *Melissococcus plutonius*

[https://etd.ohiolink.edu/pg\\_10?::NO:10:P10\\_ETD\\_SUBID:183256](https://etd.ohiolink.edu/pg_10?::NO:10:P10_ETD_SUBID:183256)

**ABSTRACT** Wax brood comb is the place where larval honey bees are reared, making it the physical heart of a honey bee colony. Generations of larval rearing darken the color of the wax and leave behind layers of excrement, silken cocoons and sometimes bacterial or fungal spores that are harmful to developing or adult honey bees. Additionally, the chemical nature of beeswax creates a sink for many compounds to be absorbed—these include pesticides and beekeeper-applied acaricides, as well as pheromones produced by larval honey bees. Finally, propolis—a dark brown antimicrobial substance collected by bees from plant buds—can also become concentrated around the rims of wax comb cells. As beekeepers become more aware of the risks caused by potential pesticide and microbe build-up, a practice known as brood comb replacement is becoming more popular in the United States. With this practice, beekeepers are

systematically removing and replacing old brood combs after several years in use. However, there is little research on the effects of old brood comb on honey bee survival or the frequency with which beekeepers should replace brood comb. Additionally, some beekeepers prefer to save old brood combs, as they are preferred by honey bee swarms that are settling into a new nest space. This thesis aims to elucidate some of the potential benefits of old darkened brood comb by recording differences in colony preference for, larval survival on, and antimicrobial activity of wax combs that have or have not been used for larval rearing. Overall, we observed no differences in colony preference for or larval survival on comb treatments that were or were not previously used for larval rearing. However, there were antimicrobial effects of extracts made from both darkened brood comb and light honey comb against a honey bee pathogen, *Melisococcus plutonius*—the bacterial agent responsible for a larval bee disease called European Foulbrood. Extracts of propolis were also found to inhibit *M. plutonius* growth, suggesting that the antimicrobial effects of wax may derive from the propolis incorporated into wax. However, the antibacterial components of wax combs are still unknown and should be studied further.

Included because of known high antibacterial effect of honey from Warré brood comb.

### Comparison of extensive v. intensive apiculture

J. Apicult. Res. 2019 , 58, 433–443.

Taric, E.; Glavinic, U.; Stevanovic, J.; Vejnovic, B.; Aleksic, N.; Dimitrijevic, V.; Stanimirovic, Z.

Occurrence of honey bee (*Apis mellifera* L.) pathogens in commercial and traditional hives

This work was aimed at the detection of the differences in the occurrence of seven bee pathogens between bee colonies kept in commercial and traditional ways. The research was conducted on 120 apparently healthy, commercially kept colonies in DB hives and 24 traditionally kept colonies in primitive, so-called trmka hives on the Pester Plateau. Brood samples were taken from all colonies to assess the occurrence of bee brood disease agents (*Paenibacillus larvae*, *Melisococcus plutonius*, *Ascospaera apis* and sacbrood virus – SBV) and adult bee pathogens (deformed wing virus – DWV, chronic bee paralysis virus – CBPV and acute bee paralysis virus – ABPV). PCR diagnostics was used in all cases, in compliance with the existing methods adopted by OIE. Concerning bee brood disease-causing agents, in commercial hives *P. larvae* (16.67% samples), *A. apis* (15.83%) and the SBV (96.67%) were confirmed, whilst in traditional hives, SBV was the only one detected (33.33%). *M. plutonius* was not found in any sample. As for adult bee diseases, in both commercial and traditional hives all of the three viruses were detected (DWV, ABPV, CBPV), but their occurrence in the former (100.00, 100.00 and 83.33%, respectively) was significantly ( $p < 0.001$ ) higher than in the latter (33.33% occurrence of each). No commercially kept colonies were free from all disease causes, while in the traditionally kept group there were 66.66% of such colonies. It can be concluded that the traditional way of beekeeping provides significantly better conditions for maintenance of bee health and their resistance to pathogens.

Oxidative Stress, Endoparasite Prevalence and Social Immunity in Bee Colonies Kept Traditionally vs. Those Kept for Commercial Purposes

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**Abstract:** Commercially and traditionally managed bees were compared for oxidative stress (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and malondialdehyde (MDA)), the prevalence of parasites (*Lotmaria passim*, *Crithidia mellificae* and *Nosema ceranae/apis*) and social immunity (glucose oxidase gene expression). The research was conducted on Pester plateau (Serbia—the Balkan Peninsula), on seemingly healthy colonies. Significant differences in CAT, GST and SOD activities ( $p < 0.01$ ), and MDA concentrations ( $p < 0.002$ ) were detected between commercial and traditional colonies. In the former, the prevalence of both *L. passim* and *N. ceranae* was significantly ( $p < 0.05$  and  $p < 0.01$ , respectively) higher. For the first time, *L. passim* was detected in honey bee brood. In commercial colonies, the prevalence of *L. passim* was significantly ( $p < 0.01$ ) lower in brood than in adult bees, whilst in traditionally kept colonies the prevalence in adult bees and brood did not differ significantly. In commercially kept colonies, the GOX gene expression level was significantly ( $p < 0.01$ ) higher, which

probably results from their increased need to strengthen their social immunity. Commercially kept colonies were under higher oxidative stress, had higher parasite burdens and higher GOX gene transcript levels. It may be assumed that anthropogenic influence contributed to these differences, but further investigations are necessary to confirm that.

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