

Issue 14 • August 2025

# NO BEES LIFE

EBA MAGAZINE



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# THE EUROPEAN BEEKEEPING ASSOCIATION WANTS TO **CONNECT AND COOPERATE!**

The EBA leadership will hold talks with the leadership of all beekeeping organizations in Europe as part of Apimondia in Denmark. The goal is to join forces and help European beekeepers together and protect the European consumer from counterfeit honey together. Only united and united will we achieve our goals when they are mostly the same.

However, we do not want to unite only all beekeeping organizations in Europe, we want to connect all scientists working in the field of bees and beekeeping. Above all, we want beekeeping organizations in Europe to cooperate even more

closely with scientists and for scientists to cooperate with beekeepers. Cooperation by everyone will bring results and I am convinced that we are capable of this in Europe in the field of beekeeping.

United work will bring results, it does not matter who is responsible for the results, the key is that the results of the work benefit bees and beekeepers!

President of the European  
Beekeeping Association  
**Boštjan Noč**







# LETTER TO THE EUROPEAN MEDICINES AGENCY

The EBA is preparing to send a letter to the European Medicines Agency (EMA) to highlight ongoing challenges in the accessibility of veterinary medicinal products (VMPs) for beekeepers. To ensure our message reflects the real experiences of beekeepers across Europe, we are kindly asking for your support. Your input will be instrumental in helping us advocate effectively for improvements that benefit beekeepers across Europe.

Please share:

- Concrete examples of the difficulties beekeepers in your country face in accessing VMPs;
- Key issues related to the treatment of colonies;
- Proposals for solutions at the European level;

Deadline: **10 August 2025**. Please send your responses to: [urska.ratajc@ebaurope.eu](mailto:urska.ratajc@ebaurope.eu)

Thank you very much for your cooperation.

With best regards,

**Dr. Urška Ratajc**

Head of the Scientific  
Committees

European  
Beekeeping  
Association



Brdo pri Lukovici 8, 1225 Lukovica, Slovenia

<https://ebaurope.eu/>



**BEES  
LIFE**

# DECISION OF THE EXECUTIVE BOARD OF THE **EBA: DR. PETER KOZMUS** CANDIDATE FOR THE POSITION OF VICE – PRESIDENT OF APIMONDIA

At its meeting held on 2 July 2025, the Executive Board of the European Beekeeping Association made the following decision:

“The European Beekeeping Association supports Dr. Peter Kozmus from Slovenia for Vice President of Apimondia and will also vote for his candidacy at Apimondia in Denmark.”

Apimondia, the International Federation of Beekeepers’ Associations, organizes the world’s leading congress on beekeeping, bringing together scientists, professionals, and associations

from all over the globe. This congress serves as a vital platform for sharing knowledge, advancing sustainable beekeeping practices, and strengthening international collaboration.

The European Beekeeping Association is proud to actively participate in Apimondia, contributing to the global dialogue on beekeeping and supporting leadership that promotes innovation, environmental responsibility, and the well-being of beekeepers worldwide.



# PERFORMANCE – BASED INCENTIVE **DECISION**

At the written meeting of the EBA Executive Board held on 11 July 2025, was passed:

“Performance-Based Incentive Decision:

A representative of the

European Beekeeping Association who successfully secures a signed sponsorship agreement on behalf of EBA, starting from 01.07.2025, shall be entitled to performance-based bonus of 10% net of the total sponsorship value.

This incentive applies exclusively to finalized and duly signed sponsorship contracts, and the bonus will be disbursed after the full sponsorship amount has been received by EBA”.

This decision aims to recognize and encourage proactive efforts in securing financial support for EBA’s mission and programs.

***EBA Executive Board***





# THE LARGEST BEEKEEPING EVENT IN EUROPE WILL BE HELD IN SLOVENIA IN 2026!

We are pleased to invite you to the Joint European Beekeeping Event, a unique gathering co-organised by B-THENET, the European Beekeeping Association (EBA) and the Slovenian Beekeepers' Association (SBA).

This three-day event will be held on 27–29 August 2026 at the heart of Slovenian Beekeeping Association in Brdo pri Lukovici, 8, 1225 Lukovica, Slovenia. It offers a valuable opportunity for knowledge exchange, collaboration, and celebration of Europe's vibrant beekeeping community.

## Event Schedule:

- **Thursday, 27 August 2026 – B-THENET Final Event** The closing event of the B-THENET project will be reserved to project partners.
- **Friday, 28 August 2026 – International Beekeeping Event** Organised by BeeLife, Apimondia, and B-THENET in collaboration with EBA and SBA, this day brings together beekeepers, researchers, policy makers, and environmental stakeholders. Discussions will focus on sustainability of beekeeping in Europe. Here, we will also present the final results of B-THENET, including the Manual for Beekeepers, a comprehensive collection of Best Practices developed across 13 European countries. The manual will be freely available for download to beekeepers.
- **Saturday, 29 August 2026 – Technical Excursions** Explore Slovenian apiaries, cooperatives and innovative practices through guided field visits and on-site demonstrations.

Come and connect with fellow professionals, share your expertise, and be part of a European movement for sustainable beekeeping!

📍 Location: Slovenian Beekeeping Association, Brdo pri Lukovici, Slovenia

✉ More information and registration details coming soon.

We look forward to welcoming you in Slovenia!



# HONEY BEE ICARUS

## TEMPERATURE LIMITATIONS OF BEES IN FLIGHT

I have often thought of bees in flight on hot days like it must be like riding a motorbike for them, the refreshing fresh air rushing past. But there's a crucial difference in that they don't have a petrol motor doing the hard work for them, so it may be more like going on an energetic run. Not so fun on a 40 degree day.

Bees are well able to regulate the temperature inside hives, and as individuals can generate heat by “shivering” their wing muscles<sup>1</sup>, but what happens when they need to use those same wing muscles and don't want to generate excess heat? How do bees fly on 40 degree days without over-

heating? That was the question Dr Jordan Glass at University of Wyoming recently set out to answer.

Other insects can mitigate heat stress by being active at dusk or night but bees need light to navigate, and need to work throughout the day to keep the hive's material needs met – especially since on hot days they require at least a liter of water per hive for evaporative cooling, and it's not going to collect itself. Reducing the frequency of wing beats can reduce metabolic heat production, but typically would be assumed to significantly reduce lift capacity, and there's little point

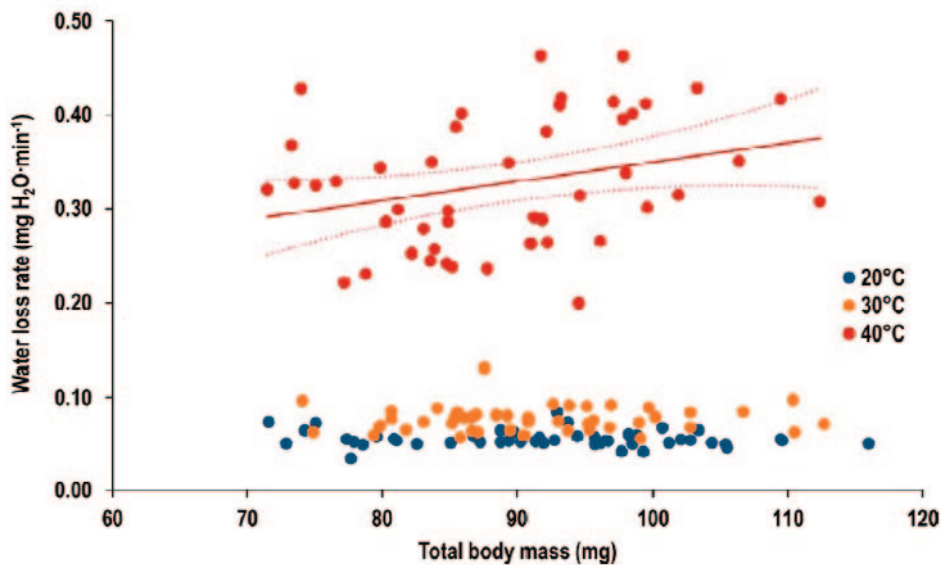


Fig. 2. Water loss rates of honey bees increased with nectar load at 40 °C, but not 20 or 30 °C air temperature. Total body mass is the mass of the unloaded bee plus the nectar load it is carrying. Each point represents an individually measured bee. The regression line and its corresponding 95% confidence limits denote a statistically significant effect of total body mass on water loss rate.

on going on a flight if you can't bring anything back. Honeybees routinely carry 20-35%, and sometimes as much as 80% of their own weight in foraged materials.

Studies have shown that bees can stay alive inside a hive in extreme temperatures (in one example a hive was surviving at regular external temperatures of 60°C on an (inactive) lava field, with the internal temperature never exceeding 36°C), but if individual bees' body temperature reaches 49-50°C they will die. If bees become unable to leave the hive and forage, then the hive will be on a crash course with running out of resources and failing, so how bees' flight performance is effected at high temperatures is worth having a look at.

Dr Glass and his colleagues set out to study exactly this. They measured flight muscle temperatures, flight metabolic rates, and water loss rates of honey bees carrying nectar in a controlled setting at 20°, 30° and 40°C. They also used high speed video to analyze wing movements at 25° and 40°C.

Wing movement (“kinematics”) in flight consists of two pertinent movements: a sweeping motion (“translational force” in the accompanying charts), and the creation of rotational vortices when rotating the wing before reversing direction. Some insects generate more lift during the sweeping stage, while others generate more on

the rotational stage. Normal flight of honeybees is about an even balance.

The first set of experiments were conducted at Arizona State University in the Sonoma Desert. They used bees from three hives maintained by the university to study the effect of air temperature on body temperatures, metabolic rates and water balance. They captured bees leaving the hive for foraging runs (who would therefore be unloaded) and weighed them, finding an average mass of 70 mg with very little variation. They then fed these bees between 0 and 45 microliters of sugar solution in a temperature controlled room.

Immediately after feeding, the bees were transferred into cylindrical flight chambers, rested for two minutes, and then encouraged to fly by shining a light at them, tapping on the cylinder or inverting it. Carbon dioxide and water content (the results of respiration) of the outgoing air from the cylinder was monitored to calculate the bees' respiration.

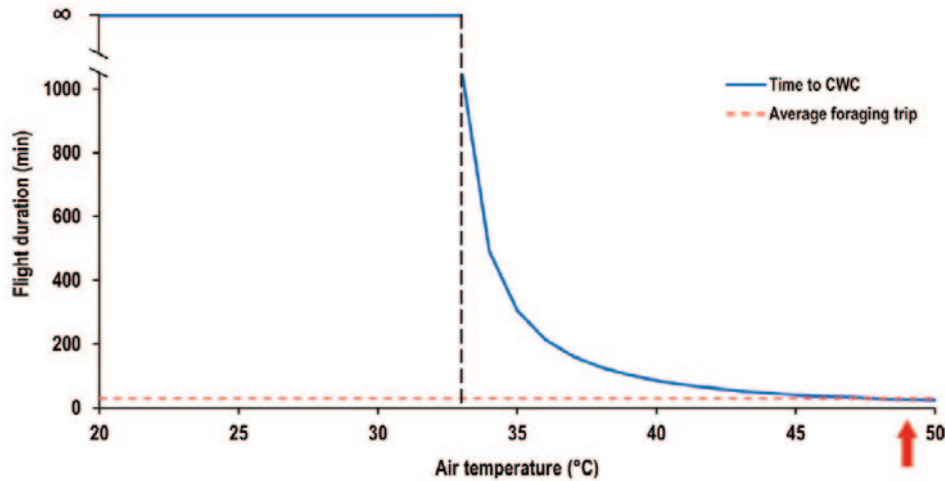
What I'm giving you here is of course the extremely simplified overview of the process laid out in the paper, see the paper itself if you particularly want details about “LI-COR LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analyzers,” and “soda lime columns” but apparently you can calculate evaporative heat loss with this data. Within two seconds of cessation of flight they then took the bees' flight muscle temperature and weighed them.



The next experiments were to look at the effect on temperature of wing motions (“kinematics”) and took place at the beautiful campus of University of California Davis located in the agricultural countryside of northern California.

They collected nectar foragers (excluding bees with loads of pollen) and weighed them. Since the unloaded weight of bees varies little from 70 mg it was assumed all weight over that was nectar cargo. These bees were placed in a temperature controlled flight chamber and their flight was analyzed with high speed cameras.

The various experiments generally showed little difference between bees flying at 20 or 30°C. At 40°C however wingbeat frequency significantly decreased (ie they're “flapping” more slowly), and stroke amplitude increased (ie, slower, bigger strokes). This reduces their metabolic rate (ie they'd feel like they're working less hard). Interestingly this did not appear to impact lift capacity. The most significant difference however was that bees don't lose much water during flight below the mid-30s, but rapidly increasingly do so beyond that. Bees as you know, can't sweat,



**Fig. 4.** The length of time an unloaded forager (average: 70 mg) can fly at a given air temperature before reaching critical water content (CWC) when flying in dry air (blue line). The red dotted line represents the average foraging trip for a honey bee (30 min; 36). The red arrow denotes the upper critical thermal limit for honey bees at rest (approximately 49 °C; 38, 39).

which we do to benefit from evaporative cooling, but they can instead regurgitate the contents of their honey stomach over their own head to accomplish the same thing.

At 40°C bees are losing a net .21 mg of water per minute. A honey bee will die if its water content reaches less than 74% of its mass, ie losing 18 mg of its 70 mg weight.



So if a bee in flight loses more than that amount in flight through either metabolic pro-

cesses or evaporation it will die. At 40°C that gives them about 85 minutes of flight time, well in excess of the average 30 minute foraging trip. It's only at 46°C that the survivable flight time drops below 30 minutes, though they may find water or nectar (which has a high water content) during their flight which will extend their flight time. However that's based on extrapolated calculations and it is not yet known if they experience serious health harm before reaching the point that would kill them, and I think it would be surprising if they don't give a very wide margin to that limit – we ourselves certainly aren't going to put ourselves in a situation nearing a few minutes or degrees of suffering a heat related death and I don't think we should expect bees to either.

The Dr Glass also notes that all these experiments were conducted in low humidity conditions, high humidity air such as one might experience in tropical parts of Australia may have a significant effect – humidity may eliminate the evaporative loss that is the current limiting factor, but then again it may also severely limit their ability to shed metabolic heat and overheating may become the limiting factor. When I kept bees in the subtropical Bundaberg area it was very often 40°C and 100% humidity, which I can tell you wasn't very pleasant as a human and I'm very curious how it would effect the bees. I hope some follow up research will look into this specifically.

I was wondering why it seemed to be the bees simply began flying more efficiently at 40°C,



—there surely must be a tradeoff?— and then I was struck by this sentence by Dr Glass:

“Bees flying at 25°C displayed high wingbeat frequencies that were independent of total body mass, suggesting that bees use a less efficient kinematic strategy when flying in cool air, perhaps to generate additional metabolic heat and warm themselves toward 39°C, the flight muscle temperature associated with maximal flight metabolic rate.”

Of course! This was a revelation – bees fly most efficiently at 39°C, they are known on cold days to shiver their wing muscles to get up to this temperature, and so when flying at less than that temperature they are essentially flying less efficiently to generate extra heat to reach that temperature! It's not that they suddenly get “magically” more efficient at and above 40°, it's that they're intentionally flying inefficiently below that.

The research conclusions seem to confirm my observation that bees really seem busiest and “happiest” on days in the upper 30s. Water seems to be the limiting factor, which is all the more argument for providing water sources to your bees on hot days, perhaps both near the

hive and if possible along their path to foraging. I thank Dr Glass for conducting this useful research and particularly for sharing it with me so I can share it with you.

*This article originally appeared in the Australasian Beekeeper June 2024*

A summary of recent research headed by Dr Jordan Glass of University of Wyoming



**Kris Fricke**

Editor of  
the Australasian  
Beekeeper

# THE AUSTRALASIAN Beekeeper

## References

- 1 Ellis, Jamie (2016) “Field Guide to Beekeeping: Colony Level Thermoregulation and the Honey Bee Dance Language” *American Bee Journal* 156:2
- 2 J.R. Glass, et al. (2024) Flying, nectar-loaded honey bees conserve water and improve heat tolerance by reducing wingbeat frequency and metabolic heat production, *Proceedings of the National Academy of Sciences*, 121(4). doi:10.1073/pnas.2311025121.
- 3 R. Dudley, C. P. Ellington, (1990) “Mechanics of forward flight in bumblebees: II. Quasi-steady lift and power requirements.” *J. Exp. Biol.* 148, 53–88
- 4 M. L. Winston, (1991) “The Biology of the Honey Bee” (Harvard University Press, Cambridge, MA).
- 5 Lindauer, M. (1955). “The water economy and temperature regulation of the honeybee colony.” *Bee World* 36, 81-92. <https://doi.org/10.1080/0005772X.1955.11094876>
- 6 H. Kovac, H. Käfer, A. Staben-thener, C. Costa, (2014) “Metabolism and upper thermal limits of *Apis mellifera carnica* and *A. m. ligustica*. *Apidologie*” 45, 664–677
- 7 J. D. Burdine, K. E. McCluney, (2019) “Differential sensitivity of bees to urbanization-driven changes in body temperature and water content.” *Sci. Rep.* 9, 1–10
- 8 J. R. Glass, J. F. Harrison, (2022) “The thermal performance curve for aerobic metabolism of a flying endotherm.” *Proc. R Soc B* 289, 20220298



# POPULATION GENETICS OF ECTOPARASITIC MITES **VARROA** SPP. IN EASTERN AND WESTERN HONEY BEES

## Abstract

Host shifts of parasites are often causing devastating effects in the new hosts. The *Varroa* genus is known for a lineage of *Varroa destructor* that shifted to the Western honey bee, *Apis mellifera*, with disastrous effects on wild populations and the beekeeping industry. Despite this, the biology of *Varroa* spp. remains poorly understood in its native distribution range, where it naturally parasitizes the Eastern honey bee, *Apis cerana*. Here, we combined mitochondrial and nuclear DNA analyses with the assessment of mite reproduction to determine the population structure and host specificity of *V. destructor* and *Varroa jacobsonii* in Thailand, where both hosts and several *Varroa* species and haplotypes are sympatric. Our data confirm previously described mite haplogroups, and show three novel haplotypes.

Multiple infestations of single host colonies by both mite species and introgression of alleles between *V. destructor* and *V. jacobsonii* suggest that hybridization occurs between the two species. Our results indicate that host specificity and population genetic structure in the genus *Varroa* is more labile than previously thought. The ability of the host shifted *V. destructor* haplotype to spillback to *A. cerana* and to hybridize with *V. jacobsonii* could threaten honey bee populations of Asia and beyond.

## Introduction

Host shifts of parasites can lead to biological invasions and result in emerging infectious diseases with devastating effects on the populations of the new hosts (Pimentel et al., 2005; Kumschick et al., 2015; Wells and Clark, 2019). A better knowledge of the drivers of host shifts in the natural distribution areas of parasites could help mitigating their negative effects, preventing future invasions (Kolar and Lodge, 2001; Woolhouse et al., 2005) and can contribute to a better understanding of the coevolution between hosts and parasites (Thompson, 1994).

Host shifts can be promoted by high parasite genetic diversity, low host specificity and by introgression between species (Longdon et al., 2014; Depotter et al., 2016; Wells and Clark, 2019), all of which can be studied using molecular tools (Criscione et al., 2005; de Meeus et al., 2007).

The Western honey bee, *Apis mellifera*, is a good model species to study host shifts. Because of the pollination service it provides and its economic importance (Klein et al., 2007; Kleijn et al., 2015), colonies of this social insect have been translocated to where beekeepers deemed appropriate and beneficial. Consequently, *A. mellifera* has been introduced to ecosystems beyond its natural distribution range and frequently exposed to parasites and pathogens never en-

countered before. In Asia, the ectoparasitic mite *Varroa destructor* successfully shifted to *A. mellifera* following its introduction into territories occupied by the Eastern honey bee, *Apis cerana*, the original host of this parasite (Rosenkranz et al., 2010). Lacking the necessary adaptive mechanisms against the parasite, most *A. mellifera* populations are unable to survive infestations, with negative consequences climaxing in colony failure within a few years (Rosenkranz et al., 2010).

Subsequently, *V. destructor* has become the most detrimental biotic threat to *A. mellifera* by negatively affecting the development of honey bee brood, on which the parasite feeds and reproduces (Rosenkranz et al., 2010), and by transmitting viruses (Wilfert et al., 2016).

This pest has led to the near eradication of wild *A. mellifera* populations in the Northern hemisphere (Le Conte et al., 2007; Jaffé et al., 2009) and to high losses of managed colonies worldwide (Genersch et al., 2010; Guzmán-Novoa et al., 2010; Le Conte et al., 2010; Nguyen et al., 2011; Smith et al., 2013) with high economical and societal costs (Kumschick et al., 2015).

Since *V. destructor* invaded Europe and the Americas in the 1970s and 1980s, an intense research activity on its biology in *A. mellifera* has been undertaken with the main aim of finding effective control methods to protect colonies (Rosenkranz et al., 2010; Dietemann et al., 2012).

Comparatively, little attention has been devoted to the interaction between *Varroa* spp. mites and their original host, *A. cerana* (Dietemann et al., 2012; Wang et al., 2018), despite the fact that several other mite haplotypes shifted host (Beaurepaire et al., 2015; Roberts et al., 2015).

Although they did not yet lead to new large-scale invasions, these new shifts show the propensity of the mite genus to generate more ecological and economic problems. Even though high genetic diversity has been shown in the genus *Varroa* (Anderson and Fuchs, 1998; de Guzman et al., 1998; Anderson and Trueman, 2000; Warrit et al., 2006; Navajas et al., 2010; Beaurepaire et al., 2015; Roberts et al., 2015), little knowledge currently exists on host specificity and their potential to hybridize, making it difficult to evaluate risks for new host shifts and in-

vasions. Indeed, previous studies in the endemic range of *Varroa* spp. rarely reported whether the mites collected were reproducing in their host brood, preventing a systematic evaluation of host specificity (see Roberts et al., 2015 for an exception). In addition, the genetic markers used to define species and haplotype distribution of these mites (i.e. mitochondrial markers), do not allow for the detection of introgression. Indeed, mitochondrial DNA is maternally inherited and only reflects maternal gene flow (Harrison, 1989), giving only a partial picture of population structure.

Even though paternal transmission can seem insignificant due to the reproductive system of the *Varroa* mites (mother mites produce one son and several daughters that mate together in the brood cells, Rosenkranz et al., 2010), recent studies showed that paternal gene flow is not negligible. In fact, reproduction can occur between inbred lineages when occupying the same cell (Beaurepaire et al., 2017a). Therefore, the use of nuclear DNA markers such as microsatellites can help completing the picture by unravelling finer levels of genetic structuring of populations than mitochondrial DNA (Beaurepaire et al., 2015; Roberts et al., 2015).

Here, we studied the population genetic structure of *V. destructor* and *Varroa jacobsonii* mites in Thailand using both mitochondrial DNA and microsatellite markers to unravel phenomena promoting host shifts.

In this country, the sympatric occurrence of the two hosts and several mite species (Warrit et al., 2006) leads to opportunities for host shifts. Yet, in the former study, a single mite was sampled per colony and a small fragment of the COI gene (328 bp) was used to determine the prevalence of mite haplotypes and species. We conducted a more intense sampling at a local scale and observed the ability of these mites to reproduce on the host they were collected from by monitoring their reproductive status.

This allowed us to increase chances of detecting phenomena that promote host shifts (e.g. drifting of mites, introgression) or host shifts per se (e.g. reproduction in a new host's brood). Surveying the distribution of mitochondrial haplotypes in the same regions as Warrit et al. (2006) more than a decade later, we also assess temporal changes in population structure. Our results

Host species	Location (region)	mtDNA			Microsatellites					
		N mites (N colonies)	N drifts (N colonies)	N colonies infested by multiple haplotypes	N mites (N colonies)	N drifts (N colonies)	N colonies infested by multiple haplotypes	N likely + less likely hybrids (N colonies)	N likely hybrids (N colonies)	N hybrids identified by Visual inspection
<i>A. mellifera</i>	Chiang Mai (North)	117 (10)	1 (1)	0	26 (7)	1 (1)	1	0	0	1
	Bang Saen (centre)	2 (2)	0	0	27 (3)	0	0	2 (1)	2 (1)	2
<i>A. cerana</i>	Chiang Mai (North)	65 (10)	8* + 1 (4)	4	34 (3)	5 (1)	1	1 (1)	1 (1)	1
	Bang Saen (centre)	3 (2)	0	0	16 (4)	0	0	1 (1)	1(1)	1
	Ko Samui (island)	20 (9)	0	0	12 (1)	0	0	0	0	0
	Phattalung (South)	18 (5)	0	0	32 (2)	0	0	6 (2)	1 (1)	12

Table 1. Sampling region, host species of origin and number of *Varroa* spp. mites genotyped for mtDNA and microsatellites. The table also indicates the numbers of mite drifts between host species and of introgression events between mite species

show that genetic structure and host specificity in the genus *Varroa* is more labile than previously thought.

We detected the occurrence of several phenomena promoting host shifts, which could represent a threat to the honey bee populations of Asia and beyond.

## Methods

### Populations, sampling

Between 2013 and 2015, 200 *Varroa* spp. mites were collected from drone brood cells of *A. cerana* in one to five apiaries from four regions of Thailand (Table 1, Fig. 1): (1) Chiang Mai (North) where *V. jacobsonii* haplotype North Thai and *V. destructor* Vietnam were reported in *A. cerana* (above 1000 m for the latter); (2) Bang Saen (Chon Buri, central Thailand) with *V. jacobsonii* haplotype North Thai; (3) Ko Samui (island) with *V. jacobsonii* Samui; (4) Phattalung (South) with *V. jacobsonii* Malay (Warrit et al., 2006). North of the Isthmus of Kra (North and centre locations), *A. mellifera* hosting the host shifted lineage of *V. destructor* Korea can be found.

A sample of 172 mites was thus collected from drone and worker brood cells of *A. mellifera* in Chiang Mai and Bang Saen (Table 1, Fig. 1). Although *A. mellifera* is occasionally kept south of the Isthmus, they do not survive there for long periods and have to be imported regularly from the North (P. Chantawannakul unpublished) and were therefore not screened in this region.

## Mite reproductive status

Upon opening of infested host brood cells, the reproductive state of mite foundresses was determined (Dietemann et al., 2013). The occurrence of at least one offspring of any sex confirmed that the foundresses were fertile, unless host developmental stage preceded oviposition. These cases, together with infertile foundresses were considered as non-reproductive. Percentage of reproductive foundresses is reported out of the total number of foundress mites found (fertile and non-reproductive). Mites were placed in 75% EtOH and frozen at -20 °C until DNA extraction.

## DNA extraction

DNA of individuals used for sequencing were extracted with phenol-chloroform (N = 193; Evans et al., 2013) and with TaKaRa lysis buffer for microorganisms (N = 32; Takara Bio Inc., Otsu, Japan). For the latter, the tubes were heated at 65 °C for 30 min and then at 100 °C for 10 min before adding 40 µL double distilled H<sub>2</sub>O. The tubes were then vortexed and centrifuged.

Ten microlitres of 2X GenStar PCR-ready mix (with Taq + loading dye), 7 µL double distilled H<sub>2</sub>O, 1 µL forward, 1 µL reverse primers and 1 µL of DNA extract was added to PCR tubes. Total DNA of mites collected in Bang Saen (n = 51) was isolated according to Beaurepaire et al.(2017a). DNA of individuals used for micro-

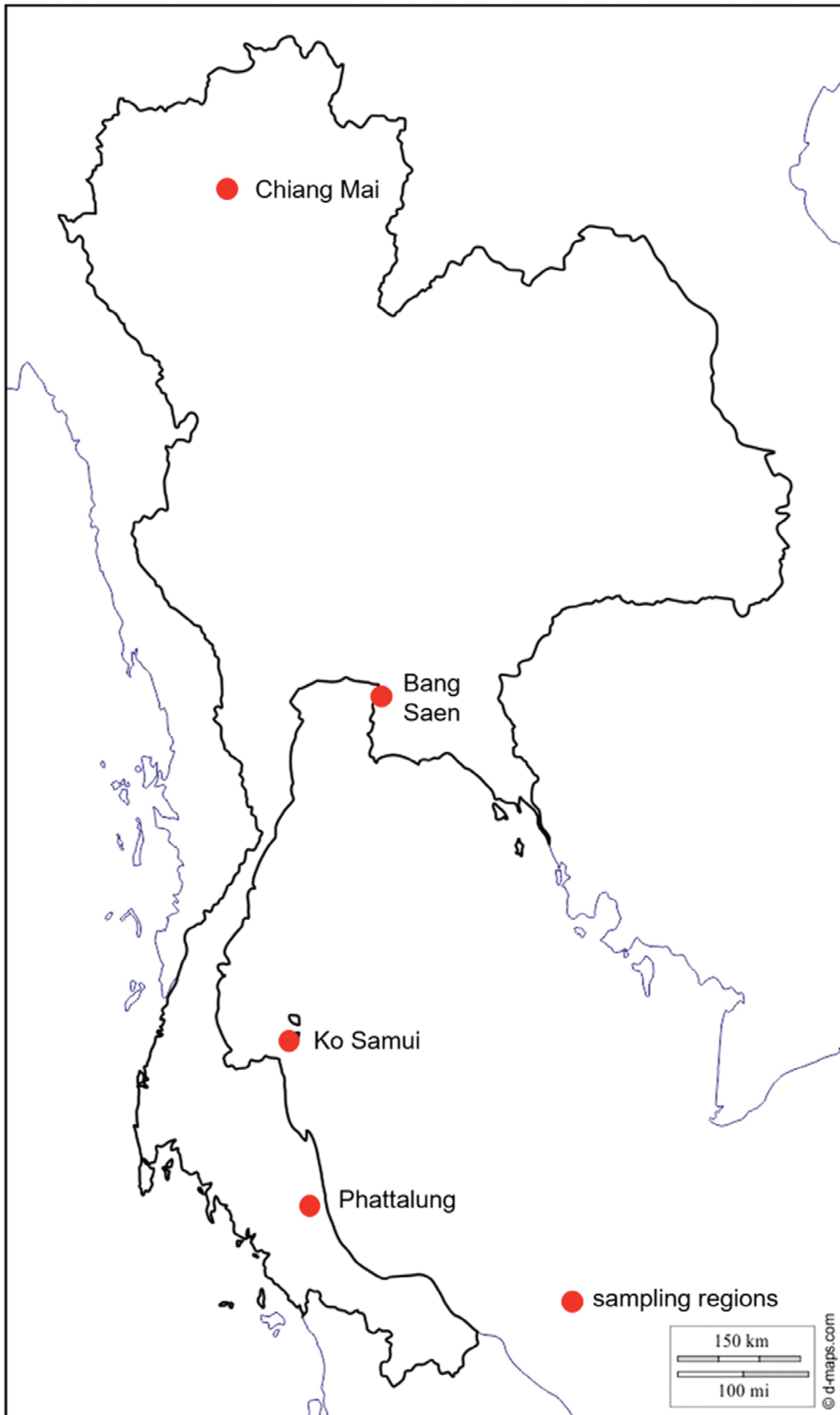


Fig. 1. Map of Thailand showing the sampling locations (see Table 1). In the text, these locations are referred to as North for Chiang Mai, centre for Bang Saen, island for Ko Samui and South for Phattalung. *Apis mellifera* colonies were screened in the North and centre and *Apis cerana* at all locations.

satellite analyses (N = 164) were extracted with Chelex: the ethanol used to preserve the mites was rinsed twice in double distilled H<sub>2</sub>O and each mite was placed individually in 100 µL 5% Chelex solution in a 96 well plate and crushed with a pestle. Finally, 5 µL proteinase K were added and the plate was placed in a thermocycler with standard Chelex cycling conditions (Walsh et al., 1991).

## PCR amplification and sequencing

PCR assays were performed to amplify regions of the cytochrome oxidase subunit I (cox1) gene of the mites sampled from the North, South and island locations (Evans et al., 2013; Table S1).

The analyses were performed by using MyTaq™ kit (Bio-line, London, UK) following the manufacturer's recommendations. Briefly, 2 µL 10-fold-diluted of the extracted DNA, 5X reaction buffer, forward and reverse primers (final concentration of 0.4 µM each) and Taq polymerase (0.63 units) were mixed in 25 µL final reaction volume. Primers given in Table S1 were used to produce 380 and 800 bp amplicons.

The PCR cycling protocols are given in Table S2. The PCR products were analysed with a 2% two-dimensional agarose gel electrophoresis. The gels were stained GelRed (Biotium, Hayward, CA, USA) for visualization under UV light. The PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Co., Düren, Germany) following the manufacturer's recommendations. Purified PCR products were commercially sequenced. Each PCR product was sequenced in both directions.

## Haplotype network analyses

A dataset including the overlapping region of the 380 and 800 bp sequences together with GenBank references (Anderson and Trueman, 2000; Warrit et al., 2006; Navajas et al., 2010) was generated (Fig. 2). Median-Joining haplotype networks of this 290 bp region were obtained in PopART with epsilon = 0 (<http://popart.otago.ac.nz>). The sequence of one representative mite per host species and region was uploaded to GenBank with the accession numbers MN179648–MN179654.

## Microsatellite DNA analyses

Varroa spp. mites collected at all locations were genotyped at eight DNA microsatellite loci (VD305, VD307, VD112, VJ292, VJ294, Vdes-01, Vdes-02, Vdes-04; Evans, 2000; Solignac et al., 2005) using the Fragment Profiler soft-

Table 2. Allele frequencies per microsatellite locus and ratio of frequencies between *V. destructor* and *V. jacobsonii*

Locus	Allele size	Species		Ratio Higher/lower frequency	
		<i>V. destructor</i>	<i>V. jacobsonii</i>		
VD305	110		5.1		
	113*	1.0	6.8	7.1	
	116		20.5		
	119		19.9		
	122	28.8			
	125*	1.0	12.5	13.0	
	128		34.1		
	131	61.5			
	134	7.7			
	137		1.1		
VD307	163	24.0			
	165**	75.0	2.2	33.4	
	167		0.6		
	169		61.8		
	171		2.2		
	173		12.4		
	175*	1.0	20.2	21.0	
	179		0.6		
	183		1.2		
VD112	133	2.1			
	135	4.3	1.7	2.4	
	137***	46.8	6.4	7.3	
	141		22.1		
	143		5.2		
	145***	46.8	22.1	2.1	
	147		16.9		
	149		3.5		
	151		3.5		
	153		15.7		
	159		1.7		
	VJ292	210		100.0	
		232	2.9		
234		97.1			
VJ294	150		1.6		
	164		9.5		
	166		19.8		
	168		4.0		
	172		12.7		
	174**	98.1	9.5	10.3	
	176		1.6		
	178*	1.9	14.3	7.4	
	180		5.6		
	182		15.1		
	186		4.0		
192		2.4			
Vdes-01	400**	41.8	1.2	34.3	
	402*	3.1	98.8	32.3	
	406	55.1			
Vdes-02	296	71.6			
	308	28.4			
	310		11.6		
	312		57.5		
	314		21.2		
	316		6.2		
	318		1.4		
	330		2.1		
	330		2.1		
Vdes-04	271***	49.0	10.9	4.5	
	273**	51.0	3.8	13.3	
	275		6.4		
	277		17.9		
	279		13.5		
	281		7.7		
	283		7.7		
	285		1.3		
	287		8.3		
	289		0.6		
	291		0.6		
	293		3.2		
	299		2.6		
301		0.6			
303		10.3			
305		4.5			

ware V. 1.2 of the MEGABACE DNA Analysis System (GE Healthcare Life Science, Buckinghamshire, England). Samples with missing information for more than three loci were excluded, resulting in a data set of 147 mites (Table 1). Hardy–Weinberg equilibrium and linkage disequilibrium tests were performed within samples for each marker using Fstat V. 2.9.3 (Goudet, 1995).

## Identification of drifters

To identify putative drifters (mites found in colonies of an atypical host, i.e. *V. destructor* Korea in *A. cerana* and *V. jacobsonii* in *A. mellifera*), an analysis not relying on a priori information was conducted with the software InStruct (Gao et al., 2007). InStruct is an alternative to the software STRUCTURE (Pritchard et al., 2000) that can handle analyses of inbred or partially self-fertilizing species, as is the case for *Varroa* spp. The number of clusters in the dataset (K) varied from 1 to 12 and 20 chains for each run were performed with 50 000 burn-in iterations and 100 000 total iterations for each chain using the Admixture model. The most likely number of clusters was then estimated manually following

the method described in Evanno et al. (2005). The results of the runs were combined with the software CLUMPP (Jakobsson and Rosenberg, 2007) and the software Distruct (Rosenberg, 2004) was used to draw the corresponding figures.

To match the genotype clusters to known mite species using the nucleotide BLAST tool on the NCBI platform (Altschul et al., 1990), a subset of 15 mites used in the microsatellite analysis were sequenced with the 929 bp COI primer from Navajas et al. (2010). See section 'Mitochondrial DNA analysis' for methodology.

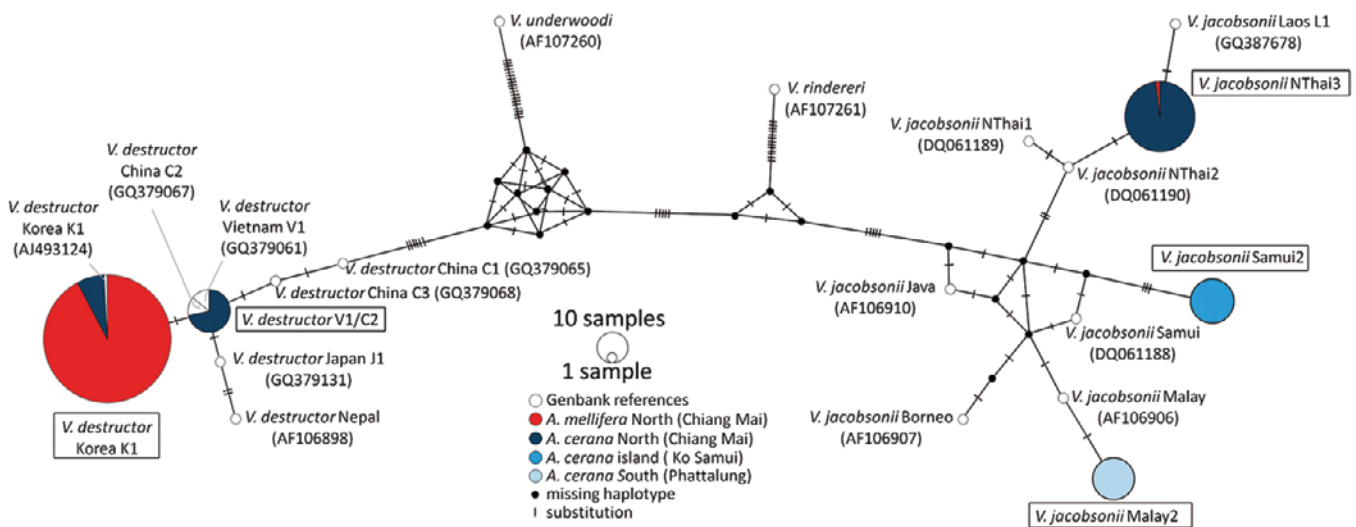


Fig. 2. Haplotype Network (Median-Joining) based on mtDNA of *Varroa* spp. sampled in three regions of Thailand (Chiang Mai, Ko Samui, Phattalung, Table 1) and from reference collections (Anderson and Trueman, 2000; Warrit et al., 2006; Navajas et al., 2010). Haplotypes detected during the present survey are highlighted with a box. Reference samples are shown without boxes and followed by their accession numbers between parentheses. Host species of origin are coded with colours: red for *A. mellifera* and blue for *A. cerana*. Location latitude is coded with shades: dark to light from north to south

## Identification of hybrids

To identify hybrids between *V. destructor* and *V. jacobsonii*, the cluster membership probabilities of each individual over the 20 chains obtained for  $K = 2$  with the software CLUMPP were compared. Individuals with a probability to belong to both clusters superior to 5% was considered as a likely hybrid. Any individual with a probability superior to 2.5% to belong to two clusters was considered as a less likely hybrid. Introgression of alleles was then confirmed based on the occurrence of heterospecific alleles, i.e. common to the two species. When the allele frequency in one of the species was 5-fold that of the same allele in the other species, we considered that the allele very likely belonged to the former species. When the ratio of allele frequencies was below five, we did not propose an origin for the allele.

## Analyses of the genetic diversity and population structure

To estimate the level of genetic diversity and population structure of the different *Varroa* taxa found in Thailand, all drifters identified by InStruct were removed from the dataset. The number of alleles (NA), allelic richness (R) and the expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ) indexes were then calculated for each mite group sampling location using the software Fstat V. 2.9.3 (Goudet, 1995).

Several estimates of genetic differentiation ( $F_{ST}$ ,  $G_{ST}$ , Dest) were calculated using GenAlex V. 6.503 (Jost, 2008; Peakall and Smouse, 2012).  $F_{ST}$  index quantifies how the reduction in gene flow among populations affects their level of heterozygosity. In addition, it reflects the variance in allele frequencies for markers with two alleles. When markers have more than two alleles, interpreting  $F_{ST}$  is more challenging (Jost, 2008). We nevertheless report  $F_{ST}$  since it is the most commonly used estimate of the reduction in heterozygosity due to population structure in population genetics studies (Whitlock, 2011). To work around this bias, we also calculated  $G_{ST}$ , which is a corrected estimate of  $F_{ST}$ , adjusted

for markers with more than two alleles (Nei, 1973). A third estimate, Jost's Dest (Jost, 2008) was calculated. It focuses on variance in allele frequencies among populations. Thus, we report the three estimates to provide complementary information on the genetic differences among the mites of the different populations as recommended in Meirmans and Hedrick (2011). In case several individuals sharing the same genotype were found in a colony, only one sample was included to estimate levels of genetic differentiation in order to avoid biasing the analysis with highly related individuals. This led to the exclusion of 25 individuals, leaving 116 for the analyses.

In addition, a pairwise distance-based AMOVA with 1000 permutations was performed for each species using the software Arlequin V.3.5.1.3 (Excoffier et al., 2005). These analyses were based on the microsatellite dataset without drifters but with putative hybrids and individuals sharing the same genotype to identify the distribution of genetic variation in each species. Finally, a Principal Component Analysis (PCA) was conducted on the same dataset to identify the main genetic clusters among the mite samples. Since we were interested in the occurrence of putative hybrids, we performed the PCA including the data of both species. The R package Adegenet (Jombart, 2008) in Rv. 3.5.2 (R core Team, 2018) was used.

## Results

### Mite distribution and reproduction

With a single exception, mitochondrial DNA sequences of the mites collected from *A. mellifera* colonies in North Thailand ( $N = 118$ ) were identified as the *V. destructor* Korean haplotype 1 (K1) (Figs 2 and 3). Twenty eight of these mites (24%) had reproduced, while the remaining mites ( $N = 89$ ) either had not reproduced or were collected from early host brood stages on which reproduction is not yet detectable. The exception was a non-reproductive *V. jacobsonii* mite in an *A. mellifera* colony (Table 1). This individual belonged to a novel haplotype that we named NorthThai3.

*Varroa destructor* K1 mites were also found in two years in ten drone brood cells of four *A. ce-*



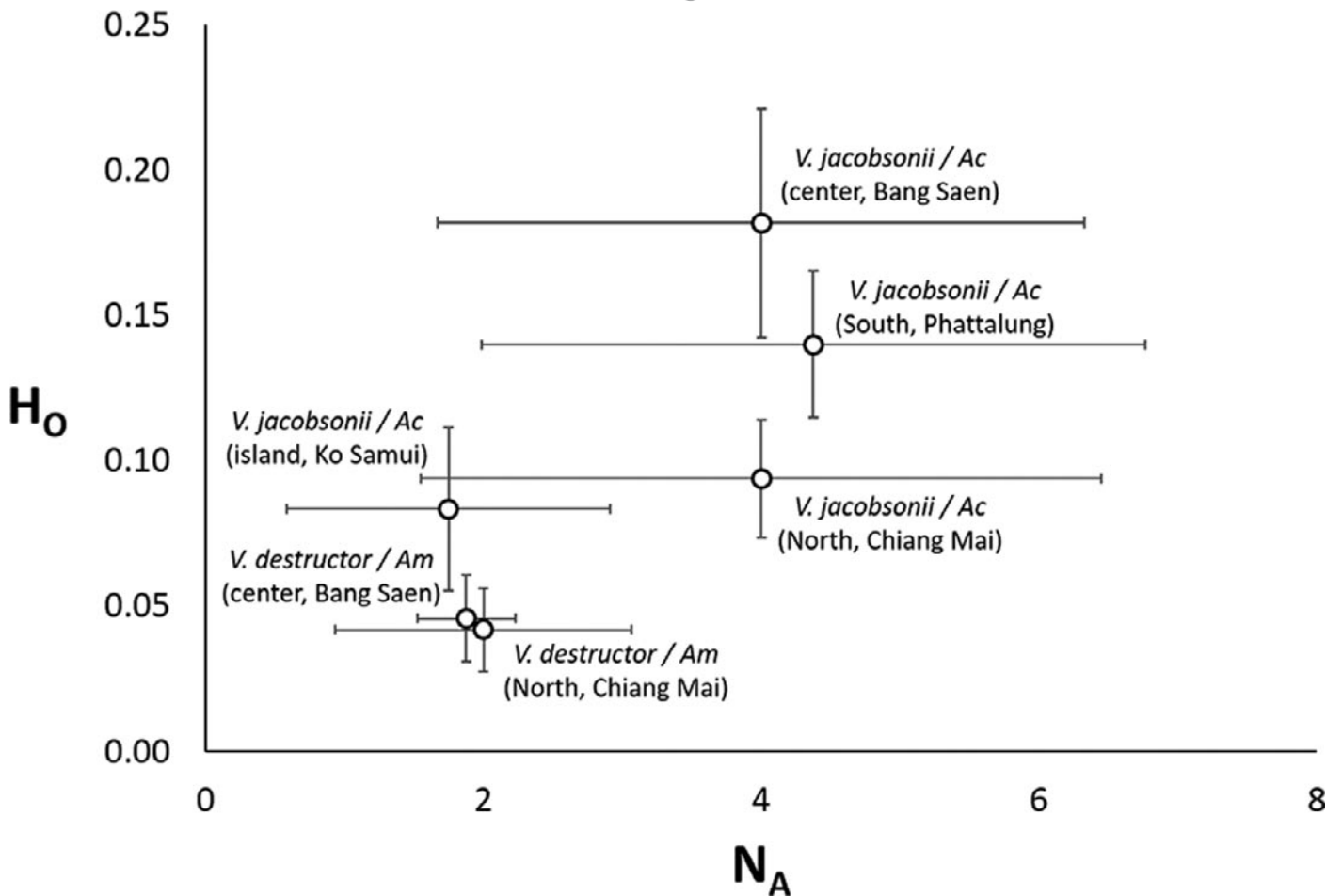


Fig. 3. Average ( $\pm$ S.D.) allelic richness vs heterozygosity in *Varroa* spp. mite populations of two host species (*Am*: *Apis mellifera*, *Ac*: *Apis cerana*) in four regions of Thailand

rana colonies in the North (Fig. 2). Eight of these mites (80%) had successfully produced offspring (Table 1). In *A. cerana* colonies screened in this region, we also found *V. destructor* of the Vietnam haplotype 1 (V1) or of the China haplotype 2 (C2, the region sequenced did not allow for discriminating between these haplotypes, Fig. 2). Three of the V1/C2 mites (60%) had repro-

duced in drone cells. The haplotype found most frequently (75%) in *A. cerana* colonies in the North was the newly described *V. jacobsonii* NorthThai3 (Fig. 2). It differed from NorthThai1 and NorthThai2 identified by Warrit et al. (2006) in two and one nucleotides, respectively, and from the Laos mite haplotype L1 in one nucleotide (Fig. 2). Twenty-six of these mites (53%) had

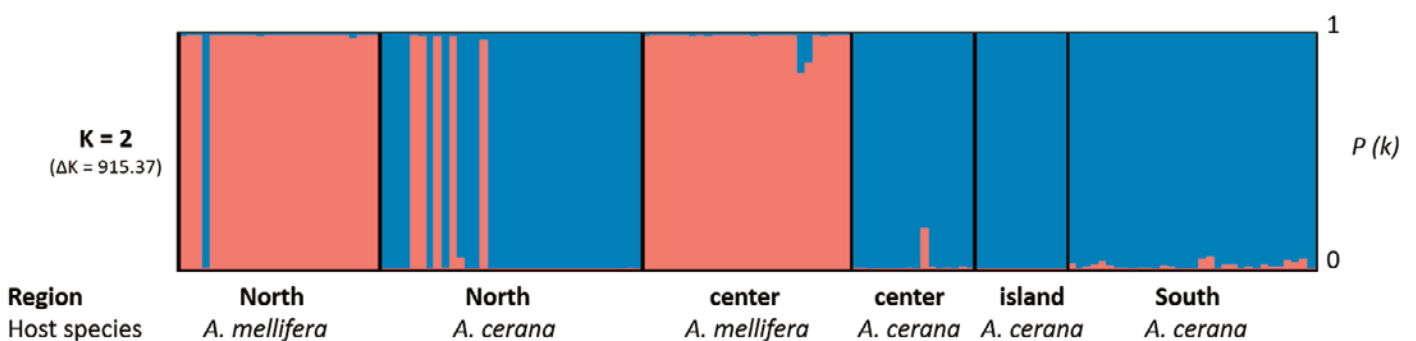


Fig. 4. Results of population structure InStruct analysis of *Varroa* spp. mites infesting *A. cerana* and *A. mellifera* in Thailand. The Y-axis represents the likelihood for each individual to belong to a genetic cluster. Each cluster is represented by a distinct colour. The X-axis shows the different individuals, their location (North, centre, South or island) and host (*Apis mellifera* or *Apis cerana*)

reproduced on drone brood. In the South, the mites found in *A. cerana* colonies differed from the *V. jacobsonii* Malay haplotype (Warritt et al., 2006) by one nucleotide (Fig. 2). We named this new haplotype Malay2. Thirteen of these mites (72%) had produced offspring in infested drone cells. Similarly, the *V. jacobsonii* mite haplotype collected from *A. cerana* colonies on the island differed from that reported earlier. We found a difference of four nucleotides and this newly reported haplotype was called Samui2 (Fig. 2). Eleven of the Samui2 mites (55%) collected from drone brood were fertile. The haplotype network inferred several haplotypes that were not sampled during our screening.

MtDNA genotyping showed that four *A. cerana* colonies in the North were infested by the two mite species simultaneously (Table 1). One of these colonies was infested with *V. jacobsonii* NorthThai3 as well as with two haplotypes of *V. destructor* (K1 and V1/C2). Each of these mite species and haplotypes reproduced on drone brood in at least one occurrence in these four colonies.

## Genetic diversity and population structure of *Varroa* spp. in Thailand

Out of the eight microsatellites we used, none of the locus pairs was significantly linked after Bonferroni correction (Table S3). These markers were polymorphic, with a range of 3–16 alleles per locus ( $8.9 \pm 4.3$ , mean  $\pm$  S.D., Table S4). The average allelic richness per locus varied from 2.1 to 6.4 ( $4.7 \pm 1.5$ , mean  $\pm$  S.D., Table S5). With the exception of low values for *V. jacobsonii* Samui2, the two genetic diversity parameters were inferior by a factor 2 in *V. destructor* compared to *V. jacobsonii* (Tables S4 and S5, Fig. 3). Given the low observed level of heterozygosity ( $H_o$ , Table S6), none of the populations sampled were at a Hardy–Weinberg equilibrium. Notably,  $H_o$  was higher by a factor 2 in *V. jacobsonii* than in *V. destructor*, again with the exception of *V. jacobsonii* Samui2 (Table S6, Fig. 3). The *V. jacobsonii* in the

North had the high allelic richness typical of the other continental *V. jacobsonii* but associated

with heterozygosity values as low as that of the island population (Fig. 3).

The analysis of population structure using InStruct showed that the most likely number of clusters in our dataset is  $K = 2$  ( $\Delta K2 = 915.4$ ). Plotting the individuals belonging to the two genetic clusters revealed a strong host specificity at most locations (Fig. 4). However, untypical host–parasite pairs ( $N = 5$  in *A. cerana* and  $N = 1$  in *A. mellifera*) were detected in the North of the country (Fig. 4). Since a portion of the individuals that were genotyped were also sequenced, microsatellite data could be associated with mtDNA haplotypes (Table S7). To do so, the nucleotide BLAST tool was used to match the individuals to known *Varroa* species. This analysis revealed that the individuals sampled in *A. mellifera* colonies shared >99.70% identity with *V. destructor* (GenBank accession GQ379056.1, 100% query cover) and that mites sampled in *A. cerana* colonies shared >98.90% identity with *V. jacobsonii* (GQ387678.1, 96–98% query cover, Table S7).

Individuals with a probability of belonging simultaneously to the two clusters above 5% (likely hybrids) were found in *A. cerana* in the North ( $N = 1$ ) and the South ( $N = 1$ ) and in both hosts in the centre ( $N = 3$ , Fig. 4, Table S8). Lowering the cut-off to 2.5% revealed five additional less likely hybrids in the southern population of *A. cerana*. Visual inspection of the genotype of these individuals confirmed the presence of shared alleles in all these individuals and identified an additional seven putative hybrids. Shared alleles occurred at one or at up to three markers simultaneously in these individuals (Table S8, Tables 1 and 2).

The PCA placed most of the putative hybrids between *V. jacobsonii* and *V. destructor*, along the axis 1, which separated the species, and which explained 30% of the genetic variance (Fig. 5). These individuals did not cluster half way between their suspected parental groups because they only showed heterospecific alleles at one to two loci and were thus closer to the parent they shared most alleles with. Four of them (corresponding to the individuals defined as likely hybrids, Tables 1 and S8) clustered outside the 95% confidence ellipses of their parent groups (Fig. 5). All other suspected hybrids (likely and less likely hybrids) lied within the ellipses. Factor 2 of the

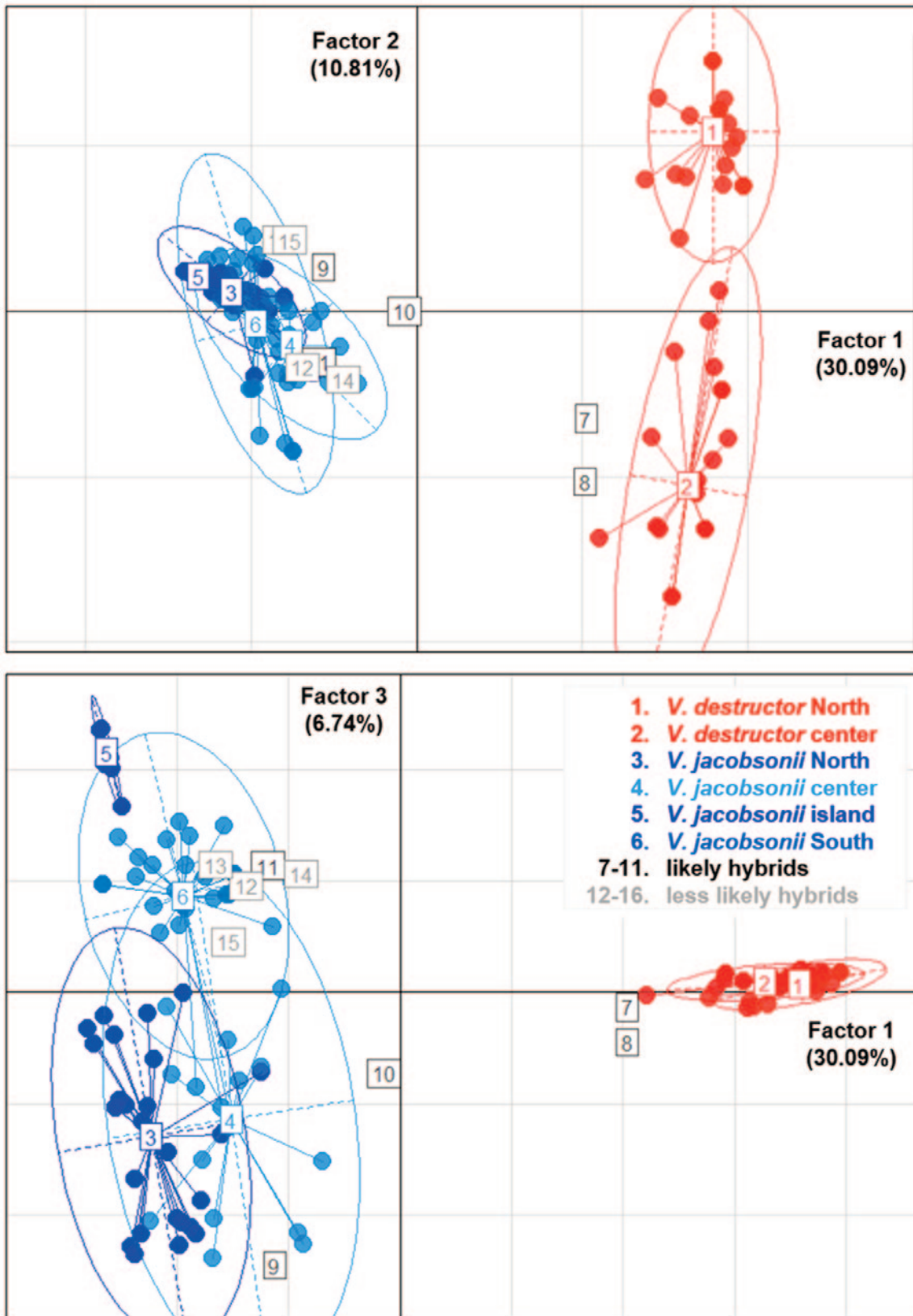


Fig. 5. Principal Component Analyses. Genetic clustering based on eight microsatellite markers of mite populations occurring in four regions of Thailand and parasitizing imported *Apis mellifera* (*Varroa destructor*, shades of red) and endemic *Apis cerana* (*Varroa jacobsonii*, shades of blue). The three factors explaining most of the variance are plotted. Percentage of explained variance is indicated on each axis. Putative hybrids identified by InStruct are indicated with numbered squares from 7 to 16. Ellipses represent 95% confidence intervals

PCA explained 11% of the variance and separated the northern from the central *V. destructor* populations. Factor 3 (7% of the variance) did not separate *V. jacobsonii* from the North and the centre of Thailand, but these two populations were separated from the island and the South populations on the third axis (Fig. 5).

The pairwise comparison of population differentiation estimates ( $F_{ST}$ ,  $G_{ST}$  and  $D_{est}$ ) showed strong significant differences between the mites parasitizing the two host species in the northern and central locations (Table S9, Fig. 6). Differences between mites parasitizing *A. mellifera* in the two regions were strong and significant (Table

S5). In mites infesting *A. cerana*, the population differentiation estimates were highest when comparing the continental populations (North, centre and South) to the island population (Table S9, Fig. 6).

The AMOVA results indicated that genetic differences among mites infesting colonies of the same location were the least important in *A. cerana* and *A. mellifera* (13.8 and 5.3%, respectively, Table 3). The highest level of genetic structuring was within colonies for mites infesting *A. cerana* (57.4%), and among locations for mites infesting *A. mellifera* (60.9%).

## Discussion

Our data confirm the *Varroa* spp. haplogroups detected previously (Smith and Hagen, 1996; Warrit et al., 2006), but the haplotypes we found differed in 1–4 nucleotides from those described earlier. The reproductive status of the sampled mites further confirmed that these haplotypes were indeed parasites of the host populations they were collected from. Spillovers of *V. destructor* to *A. cerana* and spillovers of *V. jacobsonii* mites to *A. mellifera* colonies were observed. Genotyping revealed infestation of single host colonies with both *V. destructor* and *V. jacobsonii* as well as with several haplotypes of *V. jacobsonii*, thereby setting the stage for hybridization, which the microsatellites indicated in up to 17 out of 147 mites genotyped.

## Distribution, reproduction and genetic diversity of *Varroa* spp. in Thailand

All haplogroups reported earlier (Warrit et al., 2006; Navajas et al., 2010) were confirmed. Yet, our choice to sequence a mitochondrial genome region common to several previous studies (Warrit et al., 2006; Navajas et al., 2010) led to compromises in mite identification. For instance, the V1 and C2 haplotypes and hence the V and C haplogroups of *V. destructor* could not be distinguished from each other using the chosen region. Since the V1 haplotype was reported earlier (Warrit et al., 2006), it seems likely that this is the *V. destructor* haplogroup, which we sampled in

the North of Thailand. Interestingly, the Japanese *V. destructor* haplogroup was not detected, emphasizing that its presence in Thailand is dubious (Warrit et al., 2006). The distribution patterns of the remaining haplogroups described by Warrit et al. (2006) were confirmed in broad terms based on reproductive data and genotyping: *V. destructor* K1 was found to infest *A. mellifera*, while *V. jacobsonii* North Thai, Malay and Samui infested *A. cerana* in the North (Chiang Mai), South (Phattalung) and on Samui Island, respectively.

The *V. jacobsonii* haplotypes detected here varied from one to four nucleotides compared to those described over a decade earlier (Warrit et al., 2006). The haplotype network indicates that the novel NorthThai3 haplotype of *V. jacobsonii* has not emerged recently because of its intermediate position between the NorthThai2 and Laos1 haplotypes, but has probably not been sampled previously. Despite the collection of a high number of mites in the same region, some previously described haplotypes (Warrit et al., 2006) were not confirmed, which could be due to a sampling bias. Indeed, the haplotype network inferred the existence of a few non-sampled haplotypes. Irrespective of their cause, the differences between studies and the high genetic diversity measured suggest that the mite population structure is dynamic in time or space. A more accurate description of the population structure and dynamics of *Varroa* spp. in their original range thus requires even higher sampling efforts.

The number of mites we sampled nevertheless allowed for the detection of unexpected host–parasite associations. Reproduction of the Korea haplotype of *V. destructor* was repeatedly observed for the first time on *A. cerana* drone brood outside of its natural range, thereby demonstrating a lower host specificity than previously suspected (see Navajas et al., 2010). Possible differences in drone brood entombing (Rath, 1999) and/or susceptibility of host worker brood (Page et al., 2016) between populations may explain why this particular lineage has not invaded all *A. cerana* populations sympatric with infested *A. mellifera*. Despite the ubiquity of imported *A. mellifera* in Asia, none of the studies investigating population genetics in *Varroa* spp. reported the invasive Korean lineage of *V. destructor* (K1) infesting *A. cerana* outside its original distribution range (Anderson and Trueman, 2000; Fuchs et

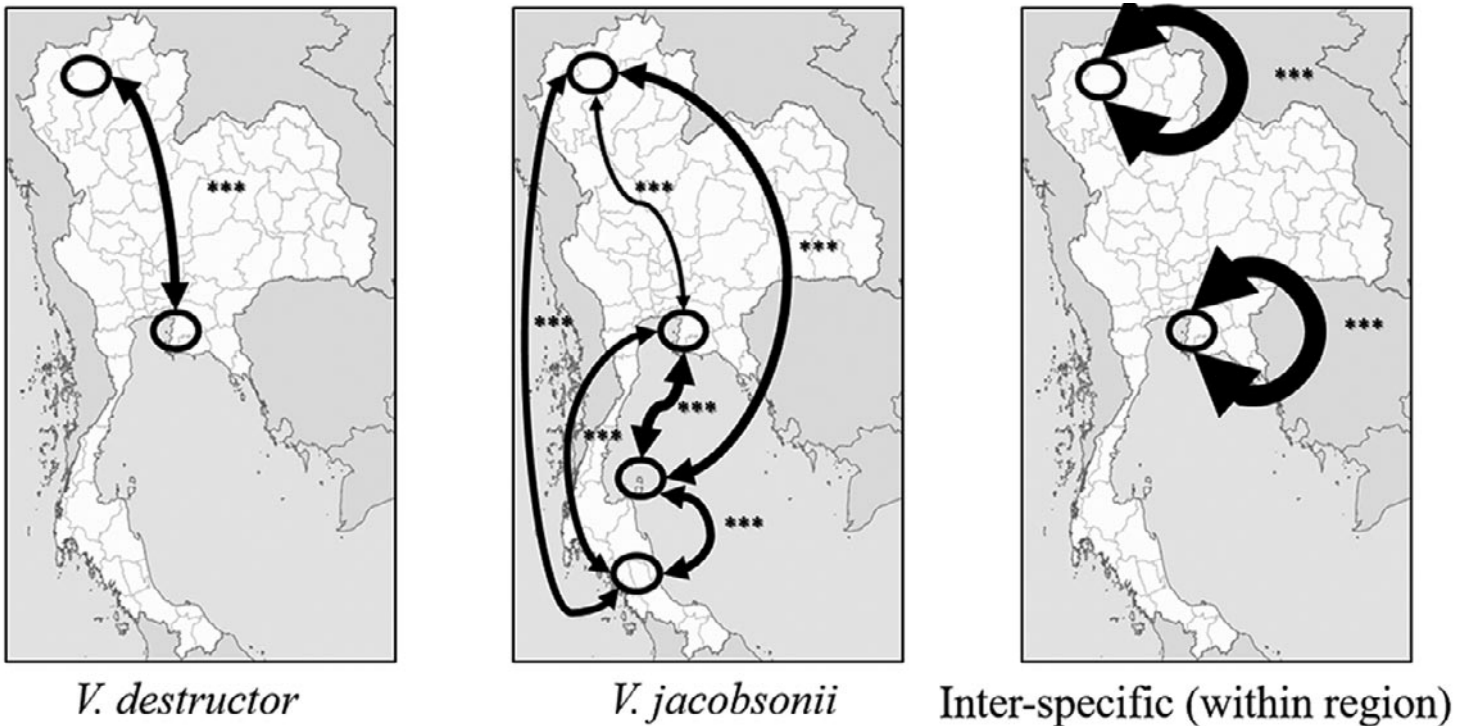


Fig. 6. Dest values between mite populations at different locations in Thailand and host species. Thickness of arrows on the maps is proportional to Dest value. Intraspecific Dest values are presented for each host species as well as for the interspecific comparison in the North and the centre. Ellipses designate sampled locations. From North to South: Chiang Mai, Bang Saen, Ko Samui, Phattalung. Statistical differences (1000 bootstrap) of Dest values between populations are denoted with asterisks (\*\*\*)  $P < 0.001$

al., 2000; Solignac et al., 2005; Navajas et al., 2010; Beaufort et al., 2015).

The number of surveys remains small and spillbacks of the *V. destructor* Korea haplotype into non native host populations of *A. cerana* could have gone undetected. Yet, the spillback of the virulent *V. destructor* lineage or its hybridization with endemic mites could have dramatic consequences for populations of *A. cerana* (Depotter et al., 2016).

Moreover, its propensity to vector a large diversity of viruses represents a threat to honey bees and other pollinators (Fürst et al., 2014; Wilfert et al., 2016).

Using mtDNA sequencing, we also detected the occurrence of a single *V. jacobsonii* mite spill-over from *A. cerana* to *A. mellifera*. The observed frequency of 1% (once in 117 cases), suggests that opportunities for host shifts by this mite species (e.g. in Papua New Guinea, Roberts et al., 2015) are not extremely rare. Whether this *V. jacobsonii* mite reproduced on its *A. mellifera* host could not be established. Nevertheless, this find-

ing is alarming and highlights the risks of host shifts by mite lineages of further haplogroups.

Drifting of mites between host species, as well as the natural sympatry of several haplotypes of mites, can lead to infestations of single host colonies and even brood cells by mites of multiple haplotypes and species, thereby setting the stage for hybridization. Indeed, such cases were detected in five *A. cerana* colonies and in one *A. mellifera* colony. In addition, the relatively high frequency of multiply infested drone cells in these populations (up to 13% of infested cells, Wang et al., in prep.) supports the idea that opportunities for hybridization can indeed be frequent.

## Putative introgression between mite species

The occurrence of several species and haplotypes in single colonies leads to the possibility of foundress mites of different taxa entering the

same host brood cell to reproduce (Beaurepaire et al., 2017a). The cohabitation of their sexually mature offspring sets the stage for hybridization. Indeed, our analysis with Instruct revealed that five likely hybrids could not unambiguously be assigned to a single genetic cluster. In complement, visual inspection of the genotypes of these individuals revealed that they carried alleles usually found on mites infesting the other host species at up to three markers (Table S8). On the PCA, some of the likely hybrids were also found outside of the 95% confidence ellipses of their group. However, given that the Adegenet PCA function incorporates missing data as averaged alleles, the other individuals (likely and less likely hybrids) carrying heterospecific alleles could not be clearly distinguished from the rest using this method. Although the presence of an identical allele in the mite species may be due to size homoplasy, this event alone does not seem sufficient to explain the patterns we observed, with the presence of shared alleles in six loci out of eight (Table S8). The large size difference with a putative parent allele strengthens our argument. For instance, we found the allele ‘175’ at locus VD307 in an individual with a dominant *V. destructor* genotype and 99.88% identity to the K1 COI haplotype (Tables S7 and S8). The closest allele found in the gene pool of *V. destructor* is 165 (75% prevalence). With a repeat motif of this microsatellite of 2 bp, at least five additions/deletions would be necessary to generate homoplasy, which seem highly unlikely.

The introgression of alleles of *V. destructor* in the gene pool of *V. jacobsonii* from the centre and the South of Thailand at some but not all loci suggests the presence of second or third gener-

ation hybrids and indicates that the two species are capable of interbreeding and of producing fertile offspring. Introgression suggests that reproductive barriers between these species are absent and questions the segregation of *V. destructor* and *V. jacobsonii* into two species. Differences in behaviour, morphology and virulence promoted the investigation of genetic divergence within the genus *Varroa* (Anderson and Fuchs, 1998; de Guzman et al., 1998; Anderson, 2000). The percentage of divergence measured resulted in the definition of *V. destructor* as a new species (Anderson and Trueman, 2000).

Yet, whether the typical biological basis for species definition is fulfilled (i.e. the absence of interbreeding and production of fertile offspring, Mayr, 1942) has never been tested. herefore, the potential of hybridization between these two mite species needs to be investigated experimentally to provide direct evidence of what our data suggest. In case it is confirmed, the relative scarcity of hybrids found to date requires an explanation. Post-zygotic isolation mechanisms, for example, have been suggested to limit the occurrence of hybrids between the Korea and Japan haplotypes of *V. destructor* (Solignac et al., 2005).

## Population structure and reproductive system

The difference between expected and observed heterozygosity (Table S6) indicates that the sampled *Varroa* populations were not in Hardy–Weinberg equilibrium, which is in line with the mites’ inbred reproductive system (Rosenkranz et al., 2010). This mating system also ex-

Mite species	Host species	Level	D.F.	Sum of square	% Variation	Significance
<i>V. jacobsonii</i>		Among locations	3	50.44	28.80	***
	<i>A. cerana</i>	Among colonies of the same location	6	16.85	13.79	***
		Within colonies	178	100.83	57.41	***
<i>V. destructor</i>		Among locations	1	42.44	60.94	**
	<i>A. mellifera</i>	Among colonies of the same location	9	9.46	5.32	n.s.
		Within colonies	89	40.50	33.75	***

Table 3. Results of two independent pairwise distance-based AMOVAs performed with the microsatellite data. Represented is the level of genetic structuring for each host species among locations, colonies and within colonies



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plains the low levels of genetic diversity, which were further exacerbated by a genetic bottleneck due to host shift (for the invasive *V. destructor*, Solignac et al., 2005) and to isolation on an island (*V. jacobsonii* Samui2; Oldroyd and Wongsiri, 2006). As a result of these bottlenecks, the number of alleles and allelic richness of these populations was inferior to that of the mites in other *A. cerana* populations. A notable inconsistency was observed for the northern (Chiang Mai) population, which showed the high range in allelic richness typical for *V. jacobsonii* (except Samui2), but a lower range of heterozygosity (similar to Samui2; Fig. 5). This suggests a higher inbreeding rate in this population, but without loss of allelic richness, of which the causes remain unknown. Overall, the range of genetic diversity of the *V. jacobsonii* populations in Thailand was similar to that found in other populations of this mite taxon (Roberts et al., 2015).

Our investigations of the population structure with microsatellite markers show that the gene flow between *V. destructor* and *V. jacobsonii* was overall low (Table S5) but may be mediated by occasional hybridization. The comparison of the different indexes of population differentiation revealed contrasting patterns in the mites infesting the two host species. The two indexes  $F_{ST}$  and  $G_{ST}$  were generally higher in *A. mellifera* than *Dest*. This trend was reversed for the mite populations infesting *A. cerana*. These discrepancies can be explained by the differences in the number of alleles and heterozygosity levels within these two groups (Meirmans and Hedrick, 2011), with *V. destructor* subpopulations being less diverse than those of *V. jacobsonii*.

The pattern of genetic structure among the *A. cerana* mite populations mostly correlated with geographic distance and isolation (Table S5, Figs 4–6). In the native host, mite subpopulations from the continent may have exchanged alleles frequently, probably as a consequence of *A. cerana* colonies migrating seasonally (Oldroyd and Wongsiri, 2006). Notably, lower levels of genetic differentiation were detected between mites from the North and the centre compared to the mites from the South (Figs 5 and 6), probably reflecting mite adaptation to the local host haplotypes (Rueppell et al., 2011). However, we found evidence of nuclear gene flow across the Kra Isthmus, which physically separates *A. cerana* Mainland and Sundaland subpopulations. These results support the hypothesis that host–parasite associ-

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ations in the Apis–Varroa system are not only due to local coevolution, but can be influenced by biogeographic history and population migration (Rueppell et al., 2011). The genetic distinctiveness of the Samui island mite population mirrors its host’s geographical isolation (see Rueppell et al., 2011). Gene flow between the continental and the Samui populations was likely interrupted 18 000 to 10 000 years ago as the sea level rose (Oldroyd and Wongsiri, 2006). Using the substitution rates proposed by Solignac et al. (2005), this timespan corresponds to a range of 3–14 substitutions on the COI gene when comparing the island with the other *V. jacobsonii* haplotypes, fitting with our mtDNA results (Fig. 2).

In accordance with the genetic differentiation estimates, the two AMOVAs revealed different patterns of genetic structuring in *A. mellifera* mites and in mites of the native host.

The gene flow of the new host is likely a consequence of human transportation of colonies, as feral colonies of *A. mellifera* do not occur in Asia (Oldroyd and Wongsiri, 2006). Although trade and the associated translocation of hosts along the country’s North-South axis (Chantawannakul, 2018) could be expected to level out population structuring in the parasite, we found high population differentiation levels in mites infesting *A. mellifera* (Figs 5 and 6).

These may be due to mite introductions of different origins and genotypes and/or due to local adaptation.

Additionally, we detected a low genetic structuration among colonies of the same location in *V. jacobsonii* and *V. destructor*, most likely reflecting that mites readily disperse among colonies (Dynes et al., 2016; Beaurepaire et al., 2017b). The analysis of genetic structure at the lowest scale (within colony) revealed that the genetic diversity between *V. jacobsonii* mite infesting the same colonies was considerable. Indeed, the *V. jacobsonii* genotypes in *A. cerana* colonies were sampled only once (Table S8). In contrast, the moderate genetic variance at this level in *V. destructor* reflects the lack of diversity of the Korea haplotype outside its natural distribution (Solignac et al., 2005). Altogether, given the peculiar patterns of Varroa population structure, varying in space, time and according to its host species, a broader sampling scheme will be necessary to

seize the extent of this parasite’s complex biogeography in Asia.

## Conclusion

Several of the phenomena known to promote host shifts have been observed in our screening of natural infestations of *A. cerana* and *A. mellifera* by *V. jacobsonii* and *V. destructor*. Genetic diversity of *V. jacobsonii* was higher compared to *V. destructor*. Spillbacks of invasive *V. destructor* mites from *A. mellifera* into *A. cerana* and spill-overs of endemic *V. jacobsonii* mites from *A. cerana* to introduced *A. mellifera* were observed. These events resulted in infestations of single colonies with both mite species and microsatellite marker based evidence suggested hybridization between *V. destructor* and *V. jacobsonii*. The relatively high frequency of these phenomena indicate risks of further host shifts, which could threaten honey bee populations of Asia and beyond.



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## References

Altschul, SF, Gish, W, Miller, W, Myers, EW and Lipman, DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410. Google Scholar

Anderson, DL (2000) Variation in the parasitic bee mite *Varroa jacobsoni* Oud. *Apidologie* 31, 281–292. Google Scholar

Anderson, DL and Fuchs, S (1998) Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera*. *Journal of Apicultural Research* 37, 69–78. Google Scholar

Anderson, DL and Trueman, JWH (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology* 24, 165–189. Google Scholar

Beaurepaire, AL, Truong, TA, Fajardo, AC, Dinh, TQ, Cervancia, C and Moritz, RFA (2015) Host specificity in the honeybee parasitic mite, *Varroa* spp. in *Apis mellifera* and *Apis cerana*. *PLoS ONE* 10, e0135103. Google Scholar

Beaurepaire, AL, Krieger, KJ and Moritz, RFA (2017 a) Seasonal cycle of inbreeding and recombination of the parasitic mite *Varroa destructor* in honeybee colonies and its implications for the selection of acaricide resistance. *Infection Genetics and Evolution* 50, 49–54. Google Scholar

Beaurepaire, AL, Ellis, JD, Krieger, KJ and Moritz, RFA (2017b) Association of *Varroa destructor* females in multiply infested cells of the honeybee *Apis mellifera*. *Insect science* 26, 128–134. Google Scholar

Chantawannakul, P (2018) Bee diversity and current status of beekeeping in Thailand. In Chantawannakul, P, Williams, G and Neumann, P (eds), *Asian Beekeeping in the 21st Century*. Singapore: Springer, 325 pp. doi: 10.1007/978-981-10-8222-1. Google Scholar

Criscione, DC, Poulin, R and Blouin, MS (2005) Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Molecular Ecology* 14, 2247–2225. Google Scholar

de Guzman, L, Rinderer, TE, Stelzer, JA and Anderson, D (1998) Congruence of RAPD and mitochondrial DNA markers in assessing *Varroa jacobsoni* genotypes. *Journal of Apicultural Research* 37, 49–51. Google Scholar

de Meeûs, T, McCoy, K, Prugnolle, F, Chevillon, C, Durand, P, Hurtrez-Boussès, S and Renaud, F (2007) Population genetics and molecular epidemiology of how to 'débusher la bête'. *Infection, Genetics and Evolution* 7, 308–332. Google Scholar

Depotter, JRL, Seidl, MF, Wood, TA and Thomma, BPHJ (2016) Interspecific hybridization impacts host range and pathogenicity of filamentous microbes. *Current Opinion in Microbiology* 32, 7–13. Google Scholar

Dietemann, V, Pflugfelder, J, Anderson, D, Charrière, J-D, Chejanovsky, N, Dainat, B, de Miranda, J, Delaplane, K, Dillier, F-X, Fuch, S, Gallmann, P, Gauthier, L, Imdorf, A, Koeniger, N, Kralj, J, Meikle, W, Pettis, J, Rosenkranz, P, Sammartaro, D, Smith, D, Yañez, O and Neumann, P (2012) *Varroa destructor*: research avenues towards sustainable control. *Journal of Apicultural Research* 51, 125–132. Google Scholar

Dietemann, V, Nazzi, F, Martin, SJ, Anderson, D, Locke, B, Delaplane, KS, Wauquiez, Q, Tannahill, C, Frey, E, Ziegelmann, B, Rosenkranz, P and Ellis, JD (2013) Standard methods for varroa research. In Dietemann V, Ellis JD, Neumann P (eds.). *The COLOSS BEEBOOK, Volume II: standard methods for Apis mellifera pest and pathogen research*. *Journal of Apicultural Research* 52. doi: 10.3896/IBRA.1.52.1.09. Google Scholar

Dynes, TL, De Roode, JC, Lyons, JI, Berry, JA, Delaplane, KS and Brosi, BJ (2016) Fine scale population genetic structure of *Varroa destructor*, an ectoparasitic mite of the honey bee (*Apis mellifera*). *Apidologie* 48, 93–101. Google Scholar

Evanno, G, Regnault, S and Goudet, J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 14, 2611–2620. Google Scholar

Evans, JD (2000) Microsatellite loci in the honey bee parasitic mite *Varroa jacobsoni*. *Molecular Ecology* 9, 1436–1438. Google Scholar

Evans, JD, Schwarz, RS, Chen, YP, Budge, G, Cornman, RS, de la Rúa, P, de Miranda, JR, Foret, S, Foster, L, Gauthier, L, Genersch, E, Gisder, S, Jarosch, A, Kucharski, R, Lopez, D, Lun, CM, Moritz, RFA, Maleszka, R, Muñoz, I and Pinto, MA (2013) Standard methodologies for molecular research in *Apis mellifera*. In Dietemann V, Ellis JD and Neumann P (eds.) *The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research*. *Journal of Apicultural Research* 52. doi: 10.3896/IBRA.1.52.4.11. Google Scholar

Excoffier, L, Laval, G and Schneider, S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1, 47–50. Google Scholar

Fuchs, S, Tu Long, L and Anderson, D (2000) A scientific note on the genetic distinctness of *Varroa* mites on *Apis mellifera* L. and on *Apis cerana* Fabr. in North Vietnam. *Apidologie* 31, 459–460. Google Scholar

Fürst, MA, McMahon, DP, Osborne, JL, Paxton, RJ and Brown, MJF (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506, 364–366. Google Scholar

Gao, H, Williamson, S and Busta-mante, CD (2007) An MCMC approach for joint inference of population structure and inbreeding rates from multi-locus genotype data. *Genetics* 176, 1635–1651. Google Scholar

Genersch, E, Von der Ohe, V, Kaatz, H, Schroeder, A, Otten, C, Büchler, R, Berg, S, Ritter, W, Mühlen, W, Gisder, S, Meixner, M, Liebig, G and Rosenkranz, P (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41, 332–352. Google Scholar

Goudet, J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86, 485–486. Google Scholar

Guzmán-Novoa, E, Eccles, L, Calvete, Y, McGowan, J, Kelly, PG and Correa-Benitez, A (2010) *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41, 443–450. Google Scholar

Harrison, RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution* 4, 6–11. Google Scholar

Jaffé, R, Dietemann, V, Allsopp, MH, Costa, C, Crewe, RM, Dall'Olio, R, de la Rúa, P, El-Niweiri, MAA, Fries, I, Kezic, N, Meusel, M, Paxton, RJ, Shaibi, T, Stolle, E and Moritz, RFA (2009) Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. *Conservation Biology* 24, 583–593. Google Scholar

Jakobsson, M and Rosenberg, NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics (Oxford, England)* 23, 1801–1906. Google Scholar

Jombart, T (2008) ADEGENET: a R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)* 24, 1403–1405. Google Scholar

Jost, L (2008) GST and its relatives do not measure differentiation. *Molecular Ecology* 17, 4015–4026. Google Scholar

Kleijn, D, Winfree, R, Bartomeus, I, Carvalheiro, LG, Henry, M, Isaacs, R, Klein, A-M, Kremen, C, Gönigle, LKM, Rader, R, Ricketts, TH, Williams, NM, Adamson, NL, Ascher, JS, Báldi, A, Batáry, P, Benjamin, F, Biesmeijer, JC, Blitzer, EJ, Bommarco, R, Brand, MR, Bretagnolle, V, Button, L, Cariveau, DP, Chifflet, R, Col-

ville, JF, Danforth, BN, Elle, E, Garratt, MPD, Herzog, F, Holzschuh, A, Howlett, BG, Jauker, F, Jha, S, Knop, E, Krewenka, KM, Le Féon, V, Mandelik, Y, May, EA, Park, MG, Pisanty, G, Reemer, M, Riedinger, V, Rollin, O, Rundlöf, M, Sardiñas, HS, Scheper, J, Sciligo, AR, Smith, HG, Steffan-Dewenter, I, Thorp, R, Tscharrtker, T, Verhulst, J, Viana, BF, Vaissière, BE, Veldtman, R, Westphal, C and Potts, SG (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications* 6, 7414. Google Scholar

Klein, A-M, Vaissière, BE, Cane, JH, Steffan-Dewenter, I, Cunningham, SA, Kremen, C and Tscharrtker, T (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B* 274, 303–313. Google Scholar

Kolar, CS and Lodge, DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16, 199–204. Google Scholar

Kumschick, S, Bacher, S, Evans, T, Markova, Z, Pergl, J, Pysek, P, Vaes-Peignat, S, van der Veer, G, Vila, M and Nentwig, W (2015) Comparing impacts of alien plants and animals in Europe using a standard scoring system. *Journal of Applied Ecology* 52, 552–561. Google Scholar

Le Conte, Y, de Vaublanc, G, Crausera, D, Jeanne, F, Roussellet, J-C and Bécarda, J-M (2007) Honey bee colonies that have survived *Varroa destructor*. *Apidologie* 38, 566–572. Google Scholar

Le Conte, Y, Ellis, M and Ritter, W (2010) *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie* 41, 353–363. Google Scholar

Longdon, B, Brockhurst, MA, Russell, CA, Welch, JJ and Jiggins, FM (2014) The evolution and genetics of virus host shifts. *PLoS Pathogen* 10, e1004395. Google Scholar

Mayr, E (1942) *Systematics and the Origin of Species*. New York: Columbia University Press. Google Scholar

Meirmans, PG and Hedrick, PW (2011) Assessing population structure: FST and related measures. *Molecular Ecology Resources* 11, 5–18. Google Scholar

Navajas, M, Anderson, DL, de Guzman, LI, Huang, Z-Y, Clément, J, Zhou, T and Le Conte, Y (2010) New Asian types of *Varroa destructor*: a potential new threat for world apiculture. *Apidologie* 41, 181–193. Google Scholar

Nei, M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the USA* 70, 3321–3323. Google Scholar

Nguyen, BK, Ribière, M, vanEngelsdorp, D, Snoeck, C, Saegerman, C, Kalkstein, AL, Schurr, F, Brostaux, Y, Faucon, J-P and Haubruge, E (2011) Effects of honey bee virus prevalence, *Varroa destructor* load and queen condition on honey bee colony survival over the winter in Belgium. *Journal of Apicultural Research* 50, 195–202. Google Scholar

Oldroyd, BP and Wongsiri, S (2006) *Asian Honey Bees. Biology, Conservation, and Human Interactions*. Cambridge, MA: Harvard University Press. Google Scholar

Page, P, Lin, ZG, Buawangpong, N, Zheng, HQ, Hu, FL, Neumann, P, Chantawannakul, P and Diemann, V (2016) Social apoptosis in honey bee superorganisms. *Scientific Reports* 6, 27210. Google Scholar

Peakall, R and Smouse, PE (2012) Genalex 6.5: genetic analyses in Excel. Population genetic software for teaching and research – an update. *Bioinformatics (Oxford, England)* 28, 2537–2539. Google Scholar

Pimentel, D, Zuniga, R and Morrison, D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States.

*Ecological Economics* 52, 273–288. Google Scholar

Pritchard, JK, Stephens, M and Donnelly, P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. Google Scholar

R Core Team (2018) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. Google Scholar

Rath, W (1999) Co-adaptation of *Apis cerana* Fabr. and *Varroa jacobsoni* Oud. *Apidologie* 30, 97–110. Google Scholar

Roberts, JMK, Anderson, DL and Tay, WT (2015) Multiple host shifts by the emerging honeybee parasite, *Varroa jacobsoni*. *Molecular Ecology* 24, 2379–2391. Google Scholar

Rosenberg, NA (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology* 4, 137–138. Google Scholar

Rosenkranz, P, Aumeier, P and Ziegelmann, B (2010) Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology* 103, S96–S119. Google Scholar

Rueppell, O, Hayes, AM, Warrit, N and Smith, DR (2011) Population structure of *Apis cerana* in Thailand reflects biogeography and current gene flow rather than *Varroa* mite association. *Insectes Sociaux* 58, 445–452. Google Scholar

Smith, DR and Hagen, RH (1996) The biogeography of *Apis cerana* as revealed by mitochondrial DNA sequence data. *Journal of the Kansas Entomological Society* 69, 294–310. Google Scholar

Smith, KM, Loh, EH, Rostal, MK, Zambrana-Torrel, CM, Mendiola, L and Daszak, P (2013) Pathogens, pests, and economics: drivers of honey bee colony declines and losses. *EcoHealth* 10, 434–445. Google Scholar

Solignac, M, Cornuet, JM, Vautrin, D, Le Conte, Y, Anderson, D, Evans, J, Cros-Arteil, S and Navajas, M (2005) The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honeybee (*Apis mellifera*), are two partly isolated clones. *Proceedings of the Royal Society Biological Sciences* 272, 411–419. Google Scholar

Thompson, JN (1994) *The Coevolutionary Process*. Chicago, USA: University of Chicago Press. Google Scholar

Walsh, PS, Metzger, DA and Higuchi, R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10, 506–513. Google Scholar

Wang, S, Lin, Z, Diemann, V, Neumann, P, Wu, YQ, Hu, FL and Zheng, HQ (2018) Ectoparasitic mites *Varroa underwoodi* (Acarina: Varroidae) in Eastern, but not in Western honeybees. *Journal of Economic Entomology* 112, 25–32. Google Scholar

Warrit, N, Smith, DR and Lekprayoon, C (2006) Genetic subpopulations of *Varroa* mites and their *Apis cerana* hosts in Thailand. *Apidologie* 37, 19–30. Google Scholar

Wells, K and Clark, NJ (2019) Host specificity in variable environments. *Trends in Parasitology* 35, 452–465. Google Scholar

Whitlock, MC (2011) G'ST and D do not replace FST. *Molecular Ecology* 20, 1083–1091. Google Scholar

Wilfert, L, Long, G, Leggett, HC, Schmid-Hempel, P, Butlin, R, Martin, SJM and Boots, M (2016) Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351, 594–597. Google Scholar

Woolhouse, ME, Haydon, DT and Antia, R (2005) Emerging pathogens: the epidemiology and evolution of species jumps. *Trends in Ecology and Evolution* 20, 238–244. Google Scholar



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## HONEY vs. REFINED SUGAR

# Could Beekeeping Hold the Secret to Combating Diabetes?

### Sweet Wisdom — or Sweet Ignorance?

In June 2025, a groundbreaking study titled Nature’s Blueprint for Sugar Metabolism offered an unexpected insight from two of the world’s tiniest sugar addicts: bees and ants. These creatures consume massive amounts of sugar — yet sidestep the devastating metabolic diseases that plague humans.

How? Their bodies harness a suite of natural compounds — especially flavonoids — to regu-

late blood sugar and protect neurons from inflammation and oxidative damage.

Despite this revelation, the medical community barely flinched.

### Refined Sugar vs. Raw Honey: A Tale of Two Sweeteners

For decades, refined sugar has been linked to chronic conditions like type 2 diabetes, inflam-



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mation, and cognitive decline. Yet it continues to dominate the global food system, often protected by industry interests and overlooked by public health policy.

Enter the honeybee — a living contradiction to that narrative.

Bees consume sugar constantly in the form of nectar, but thanks to the flavonoids naturally present in honey and pollen, they maintain metabolic balance and strong cognitive function. These compounds — such as quercetin, kaempferol, and pinocembrin — not only regulate blood glucose but also shield the brain from sugar-related oxidative stress.

This raises a simple but powerful question: Why aren't we learning from the bees?

## The Beekeeper's Gold: Honey as Nature's Glucose Regulator

Flavonoids found in honey — long respected in the world of beekeeping — are now being recognized by science for their antioxidant, anti-in-

flammatory, and antidiabetic properties. Research shows they can:

- Balance sugar metabolism;
- Protect neurons from glutamate-induced damage;
- Reduce oxidative stress linked to aging and diabetes.

For beekeepers, these qualities are no surprise. Raw, unprocessed honey from healthy hives has always been more than a sweetener — it's a natural pharmacy, handcrafted by bees, with therapeutic potential that modern medicine is only beginning to appreciate.

## Children, Sugar, and a Growing Crisis

While the diabetes epidemic worsens among children — largely driven by sugary beverages and refined sweeteners — honey offers an alternative. Unlike refined sugar, honey contains micronutrients and bioactive compounds that may help counteract some of the metabolic harm.

Perhaps it's time we stop banning all sugars in schools — and start replacing the bad ones



with bee-derived, biologically supportive alternatives.

## Beekeeping: An Overlooked Tool in Public Health

Modern research now shows that flavonoids in honey — particularly quercetin and pinocembrin — protect insulin-producing  $\beta$ -cells and enhance insulin sensitivity. This positions beekeeping not just as an agricultural pursuit but as a public health ally.

While pharmaceuticals dominate the diabetes market, nature — and the beekeepers who tend to her — may already hold a more accessible solution.



## What the Science Shows

Honey flavonoids have been found to:

- Lower fasting blood glucose;
- Improve lipid profiles;
- Reduce inflammation and oxidative stress.

Clinical trials show that quercetin, when used as a supplement, improves insulin sensitivity and lowers HbA1c — a key marker in diabetes control.

And yet... this hasn't made it into mainstream medicine.

## Why Isn't Honey in the Guidelines?

Despite promising evidence, honey flavonoids are largely ignored in clinical diabetes care. Possible reasons?

- Lack of awareness among physicians;
- Preference for pharmaceutical models;
- And simply: bees don't bill insurance companies.





## Flavonoids in Honey: The Beekeeper's Toolkit

Some of the most well-researched flavonoids in honey include:

- Quercetin – Regulates blood sugar, reduces inflammation
- Kaempferol – Improves insulin sensitivity
- Pinocembrin – Protects from oxidative stress and AGE damage
- Caffeic acid, Gallic acid, Ferulic acid – Impact glucose metabolism, reduce oxidative stress
- Syringic acid, p-Coumaric acid, Luteolin – Antioxidant, anti-inflammatory, improve insulin response

## Conclusion: Beekeepers at the Heart of Public Health

Perhaps it's time we reframe beekeepers not just as producers of honey — but as guardians of a potent, natural medicine. Honey isn't just tradition. It's evidence-based, bioactive, and relevant to the chronic diseases of the 21st century.

With diabetes on the rise and health systems under strain, it may be time to think differently. Or maybe, to think more like a beekeeper.

But beekeepers around the world already know what the research confirms — honey is more than just sweet. It's functional, biochemical, and healing.

## Time to Let Bees Guide the Future of Medicine

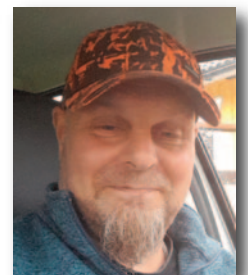
As type 2 diabetes becomes one of the great health challenges of our time, we must look beyond synthetic treatments and consider what nature already solved.

Supporting beekeeping, protecting pollinators, and integrating honey into preventive health-care aren't just ecological choices — they're medical ones.

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# GOOD HEALTH STARTS WITH **GOOD** **SLEEP, AND HONEY** **HELPS ENSURE IT**

I greatly enjoyed the two articles by Fabian Lindhe and Mike McInnes in the previous issues of No Bees No Life, which highlighted honey's contribution to energy metabolism and its role in preventing obesity. With this article, I hope to add another piece to the puzzle: how honey supports restful, restorative sleep and helps prevent metabolic stress.

In today's challenging environment—where the beekeeping sector is under siege from adulterated and fake honeys—beekeepers should not only press authorities for stronger protections but

also actively educate consumers. People need to understand why real honey is vital to their health, how to choose it, and where to buy it.

## The Link Between Metabolic Stress, Obesity, and Sleep

The brain's primary energy source is glucose, supplied from glycogen stored in the liver. Glycogen is a polysaccharide made from long chains of glucose molecules. While muscles







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store glycogen exclusively for their own use, the liver's glycogen reserves serve the entire body, especially the brain, heart, kidneys, and red blood cells. However, the brain itself can store energy for only about 30 seconds. If glucose supply fails, the body enters crisis mode.

In adults, the liver typically stores 70–100 grams of glycogen. However, when a meal is eaten 2–3 hours before sleep, much of this glycogen is already depleted by the time sleep begins. Since the brain consumes approximately 10 grams of glucose per hour during sleep, the liver's reserves can be exhausted within a few hours. Once glycogen runs low, the brain triggers an emergency response: hormones (GRH, ACTH) signal muscle tissue to break down proteins into amino acids, which the liver converts into glucose—a process known as gluconeogenesis.

This response demands the release of stress hormones, primarily adrenaline and cortisol. Chronically elevated cortisol suppresses insulin, disrupts glucose metabolism, and over time leads to insulin resistance, high blood sugar, obesity, type 2 diabetes, cardiovascular disease, and neurological disorders. Metabolism is further dis-

rupted, with increased circulating free fatty acids and cholesterol.

## Honey as a Nighttime Remedy for Metabolic Stress

Consuming 1–2 tablespoons of honey before bedtime can prevent this chain reaction. Honey supplies glucose in a controlled, steady manner. Due to its balanced glucose and fructose content, honey raises blood glucose moderately, avoiding spikes. Fructose plays a key role by enhancing liver enzymes (glucokinase and glycogen synthase), converting glucose efficiently into glycogen and replenishing liver stores before sleep.

Unlike large doses of pure glucose, honey's gentle glucose rise prompts only a moderate insulin release. This, in turn, facilitates tryptophan's entry into the brain, where it converts into serotonin—a neurotransmitter that promotes relaxation.

As night falls, serotonin transforms into melatonin, the hormone responsible for inducing sleep by lowering body temperature, slowing the heart rate, and calming respiration.

Melatonin also slows further insulin release, allowing glucose to remain in the blood longer and ensuring a steady energy supply to the brain throughout the night. Following this, growth hormone (somatotropin) and other repair hormones are released, supporting tissue regeneration, muscle maintenance, fat metabolism, and brain recovery.

The key benefit: adequate glycogen stores and stable blood glucose prevent nighttime cortisol and adrenaline surges, reducing metabolic stress and its long-term health consequences.

## Not All Fructose Is Equal: Honey vs. Industrial Sweeteners

Fructose, when consumed together with glucose—as found naturally in honey, fruits, and vegetables—supports glycogen formation and glucose stability. However, excessive intake of free fructose, particularly from high-fructose corn syrup (HFCS, HFIS), invert syrups, and adulterated honeys, bypasses normal metabolic pathways. This free fructose is rapidly converted into trioses, which feed directly into fat production cycles, contributing to fatty liver disease, liver cirrhosis, obesity, and type 2 diabetes.

In short: the fructose in natural honey is health-protective, while the free fructose in processed sweeteners is health-damaging.

## Conclusions: Honey for Healthy Sleep and Metabolism

Uninterrupted, restful sleep supports memory, emotional balance, detoxification, weight

control, hormone regulation, immune strength, and balanced metabolism. In contrast, poor sleep fosters fatigue, low energy, concentration difficulties, metabolic imbalance, and increased risk of heart, kidney, and liver problems.

To optimize sleep and avoid metabolic stress:

- Keep a regular daily routine.
- Reduce screen exposure before bedtime.
- Avoid eating 2–3 hours before sleep.
- But most importantly: consume 1–2 tablespoons (20–40 grams) of real honey shortly before sleep.

Honey's balanced fructose/glucose composition, combined with its natural enzymes and other components, replenishes liver glycogen and ensures a steady glucose supply to the brain throughout the night. This prevents the breakdown of muscle proteins, blocks the release of stress hormones, and supports healthy, restorative sleep.

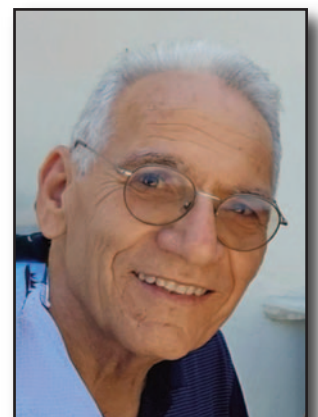
This simple, natural practice not only promotes good sleep but also protects long-term metabolic health.

This article was inspired by the excellent book by Dr. Ron Fessenden, *The New Honey Revolution: Restoring the Health of Future Generations* (ISBN 9781498400671)—a must-read for everyone interested in honey's role in human health.

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# THE **USE** OF POLLEN, ROYAL JELLY AND WAX

## Pollen

Pollen was described by the ancient Egyptians as a life-giving powder. In ancient Greece, it was believed that clusters of pollen, brought to the hive by bees, were made of wax. "It is similar

to wax in its hardness, but in reality, it is bee bread", wrote Aristotle in his book *Historia animalium*.

Hippocrates was equally confident about the healing powers of pollen, prescribing it several times to his patients. The denomination "bee





manner of pollination also depends on the size of pollen grains.

Pollination can happen in several different ways. Plants that are pollinated by the wind are called anemophilous plants. The pollen of anemophilous plants is tinier, lighter, and dry, which makes it possible for it to be carried by the wind. Plants that are pollinated by insect intervention are called entomophilous plants. The grains of pollen found in entomophilous plants are rough and sticky; they form tiny clumps and cling on insects. Flowers of entomophilous plants come in different shapes, and that is why there are differences in pollen quantities, even among entomophilous plants. In pollinating these plants, bees play a primordial role. They participate in the pollination of about 40,000 plant species; their activity in nature puts them in an indispensable role in maintaining biological diversity and in pollinating different crops.

The bodies of bees are covered in tiny hairs; when the bee sits on the flower, pollen clings on to these hairs. Bees then glue together the collected grains of pollen using saliva, nectar, or honey from their honey sacs. In this process, they also enrich the pollen with their own enzymes. During flight, bees clean themselves by putting the collected pollen into the structure for transporting pollen called pollen baskets that can be found on their hind legs. During every flight, a bee brings from 16 to 24 mg (approximately 3 to 4 million grains) of pollen back into the hive, which represents one tenth of her own weight. Pollen is collected by honey bees. The distances they can fly to collect pollen are longer than the distances they fly to collect nectar, since they need less time per plant to collect it, in comparison with collecting nectar; besides, a pollen load weighs less



bread” stuck for several centuries. The name “pollen” (Latin for “fine powder”) was first used by John Ray in his book *Historia plantarum* (1686). Pollen only began being used more regularly in people’s nutrition after World War II, when beekeepers thought of and manufactured pollen traps to capture pollen.

Pollen is characteristic of each individual plant species in flower and can be understood as a sort of fingerprint of each individual plant, which makes it so unique. It is created in four elongated pollen sacs in the so-called anther, which is the lower, widened part of the flower’s reproductive organ called the stamen. When the anther matures, it opens and millions of pollen grains get released on the surface of the stamen. The flowers of plants contain different quantities of pollen grains.

As plants are different, so are pollen grains. They differ in shape, colour, and size. The

than a nectar load. For the honey bee colony, pollen represents the main source of proteins, fats, vitamins, minerals, and other ingredients that are vital for bee survival. A honey bee colony uses from 30 to 50 kilograms of pollen per year. Bees need pollen in order to bring up their brood and to develop.

Bees bring pollen into the hive in pollen baskets on their legs. Beekeepers have created special devices called pollen traps to capture fresh pollen. A pollen trap is made out of tiny holes through which a bee, carrying a pollen load, needs to squeeze itself in order to get to the hive. While it is squeezing through the tiny holes, pollen is collected from its legs.

Another possibility is that bees bring pollen into the hive and load it into comb cells, filling them up to two thirds and filling the remaining third with honey, thus preventing pollen from going bad. Pollen stored in that manner does not come into contact with oxygen, which causes it to start fermenting and leads to development of bacteria excreting lactic acid, which is a characteristic ingredient of pollen stored in this way. Beekeepers also call this kind of pollen “bee bread”.

While honey represents a source of energy for the honey bee colony, pollen is their main source of other important nutrients such as proteins, minerals, fats, and other substances. The presence of these ingredients proves that pollen can also be used for human consumption. Since pollen is mostly a mixture of pollens from different flowers, the contents of their nutritional components also differ. Pollen that bees have only collected on one type of plant is called monofloral pollen, which consequentially contains a constant, i.e. a more equally distributed compound of biologically active ingredients. However, beekeepers usually produce mixed (polyfloral) pollen, since it is collected by bees as such.

Pollen comes in a variety of colours, from white to black, but is usually yellow, orange, or yellow-brown, and shaped in the form of spherical lumps of different sizes. Its flavour and its smell differ from sweet, sour, bitter, to sharp, depending on its botanical provenance.

It is mostly used as a foodstuff or as a food supplement. Captured fresh pollen can be eaten fresh or dry. The process of drying diminishes its water content, which makes pollen last longer. Fresh pollen, however, is extremely perishable



**BEES  
LIFE**



and needs to be kept in a refrigerator or in a freezer. Thus, fresh pollen keeps its original quality and is easily digested in comparison to dry pollen. Human beings can also easily digest bee bread. The presence of microorganisms from *Lactobacillus* and

*Bifidobacterium* genera that take part in the fermentation process, as well as ferment creation (vitamin K), enhance the nutritional value of pollen stored thusly. Pollen can be eaten in several different ways. Usually, it is taken in combination with other bee products. If it is ingested without water, it is recommended that we chew it and mix it with our saliva before swallowing. Even better, we can soak pollen in a glass of water, juice, yoghurt, or honey solution before ingesting it, or we can eat it with fruit. When in contact with a liquid, grains of pollen dissolve and are thus easier to digest; what is more, bioactive ingredients contained in pollen become more available for our bodies to use. In short, the pollen that we eat should be as fresh as possible, be stored accordingly, and come from a familiar environment; as to how we should ingest it, it all depends on personal preferences. It is recommended that we be checked for potential pollen allergies before taking it for the first time. It is important to start consuming pollen in smaller quantities that can then be increased daily. When bees bring pollen to the beehive, they add nectar and excrements of their glands with enzymes to it, which contributes to changing its structure and neutralises allergens.

However, an allergic reaction can nevertheless be provoked in people that are already allergic to pollen. If such issues should be experienced, it is important to stop consuming pollen and consult a medical specialist, if needed.

The recommended quantities of daily intake of pollen are one big spoon of pollen (15 g) at least half an hour before a meal; some people also eat two spoons per day, or consume it once or twice per year for a longer time period (between 3 weeks and 3 months) when summer turns to autumn and winter turns to spring. Available literature does not indicate that consuming daily quantities of pollen larger than the ones indicated above could cause any issues (with the exception of allergies).

Since the microscopically small grain of pollen is surrounded by a strong cellulose en-

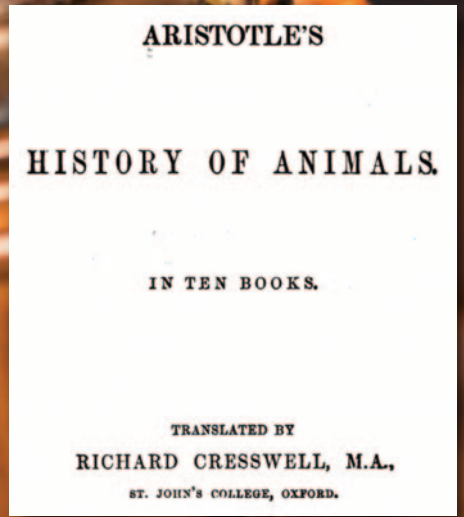
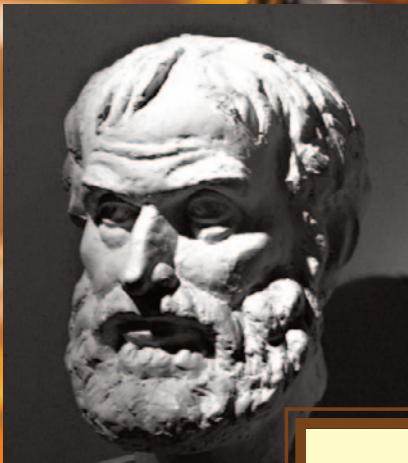
velope, one hundred percent of its nutrients can never be used by the human body (it also depends on the manner of storage, pollen processing, and botanical type of pollen). If all of its nutrients could be used, 35 grams of pollen a day would suffice to satisfy all our nutritional requirements. Pollen consumption is therefore also recommended for vegetarians; by consuming pollen, they can satisfy their daily requirements of essential amino acids that are usually mostly found in foodstuffs of animal origin.

Experts are also examining pollen to be used in certain medical conditions. Among the biologically active ingredients of pollen are also flavonoids and phytosterols that are attributed antioxidant, anti-inflammatory and antimicrobial properties.

On the basis of animal studies, it has been discovered that pollen consumption supposedly ameliorates conditions such as anaemia, arteriosclerosis, osteoporosis, and even allergies.

Pollen can also be used for animal consumption as an addition to fodder used for chickens, pigs, birds, bats, etc. Its use also extends to the

# BEES LIFE



Pollen was described by the ancient Egyptians as a life-giving powder. In ancient Greece, it was believed that clusters of pollen, brought to the hive by bees, were made of wax. "It is similar to wax in its hardness, but in reality, it is bee bread", wrote Aristotle in his book *Historia animalium*. Hippocrates was equally confident about the healing powers of pollen, prescribing it several times to his patients.

cosmetic industry, since it is used as an ingredient in nutritious creams and skin masks.

It is safe to say that pollen is the perfect food for human beings, since it contains numerous ingredients beneficial to the functioning of the human body.

## Royal Jelly

Royal jelly is a special ingredient secreted by young worker bees. Royal jelly is produced in their hypopharyngeal glands that are found in the head of the bee, right next to its brain. Royal jelly represents a source of food for the bee brood and for the queen bee. In the first three days, bees use it to feed the entire brood; later, it is only used to feed the queen bee that feeds on it for its entire life. Royal jelly has a gelatinous structure and is usually a non-homogenous product, since it can contain insoluble grains of different shapes and sizes, of off-white to pale yellow colour, and of somewhat acidulous to sharp smell and flavour.

The most common ingredient of royal jelly is water (60–70%). Its main dry ingredients are carbohydrates, proteins, amino acids and fats. Royal jelly also consists of smaller amounts of vit-



amins and minerals. Among fatty acids found in royal jelly, the most commonly represented one is 10-hydroxy-2-decanoic acid (10HDA): besides having antibacterial properties and functioning as an inhibitor of the growth of mould and bacteria, it is also the indicator of the freshness of royal jelly and its authenticity.



Because of its high water content, royal jelly demands special attention when it comes to storage, since it is an extremely perishable foodstuff. Royal jelly should be stored in the refrigerator or, even better, in the freezer; when it is stored thusly, its quality does not diminish.



# BEES LIFE



Royal jelly can be used in the nutrition of both healthy and ill people and also children. In healthy people, consuming royal jelly contributes to maintaining psychical and physical fitness, undertaking strenuous activities with less effort, and delaying ageing processes. The recommended daily dosage of royal jelly is from 100 to 300 milligrams of fresh royal jelly. We should consume it in the morning, on an empty stomach, by putting it under our tongue and waiting until it absorbs slowly.

Consuming small amounts of royal jelly as an addition to our nutrition is recommended for everyday use. We can consume it on its own or

Royal jelly can be used in the nutrition of both healthy and ill people and also children. In healthy people, consuming royal jelly contributes to maintaining psychical and physical fitness, undertaking strenuous activities with less effort, and delaying ageing processes.

in combination with bee products or other food-stuff. For certain medical conditions, the recommended daily dose of ingested royal jelly can be increased.

Royal jelly is also used in the cosmetics industry. As an ingredient, it can be found in different body care products, such as various face creams, body creams, makeup products, hair shampoos, etc.

## Beeswax

Beeswax is a substance that worker bees secrete from their wax glands that can be found on the side of their bodies next to the belly. Bees use beeswax to build combs where the queen bee





# BEES LIFE

lays eggs and where bees store honey and pollen.

While young (virgin) beeswax is almost white, colorants from pollen and propolis give it a somewhat more yellow colour. Its main compositions are mostly esters from higher fatty acids and alcohol. It has been used since time immemorial and is mostly known for being used in making candles, wax sculptures, and soaps, embalming bodies, painting, etc.

In the food industry, wax is used as a food preservative (E 901). It is also an ingredient in coatings and glazes for cakes, fruits, nuts, and chocolate, a carrier of food additives and colorants, and an ingredient in chewing gums. It can also be used in home cooking for coating baking pans before baking. Beeswax is completely indi-

gestible and has no caloric value for human beings.

References are available on the following website: <https://beebooks.si/en/>.

**Nataša Lilek**  
Slovenia

*The text was printed in the Slovenian book "No bees, no life" in 2017.*



# WikiBeedia.eu

## A MULTILINGUISTIC ENCYCLOPEDIA ON BEES AND BEEKEEPING

### Abstract

We implemented an online encyclopedia about beekeeping which aims to connect beekeepers and scientists as both groups can profit from each other's knowledge and expertise. The multilinguistic platform is meant to be used by everybody interested in bees and beekeeping. The wiki-approach is dependent on multi-actors contribution, with apidologists and beekeepers leading the effort. We need diversified knowledge from different regions and especially native speakers to translate and edit articles. To enable this, we embedded an instructions page in our homepage, which explains how to write, edit and translate articles. To make WikiBeedia easier to navigate we divided the articles in six categories. The community-driven WikiBeedia is meant to grow and sustain itself long-term!

### WikiBeedia.eu – Curated Knowledge for Everyone

Contemporary beekeeping requires diverse skills, like disease recognition, knowledge of hive management practices, technical terms, and forage plants, to name just a few. Following best practices can significantly reduce colony losses and make beekeeping more profitable (Kulhanek et al., Citation2021; Tubene et al., Citation2023), educated beekeepers experience lower colony losses (Jacques et al., Citation2017). The flow of science-based information from experts or knowledge generators, adapted to recent conditions in times of climate change, to users (beekeepers) is crucial. This is more complicated than one can imagine, since old habits are often firmly established. Researchers and advisors in agriculture face general challenges in the dissemination of

science-based information within agricultural extension, technology adoption and knowledge transfer (Aker, Citation2011).

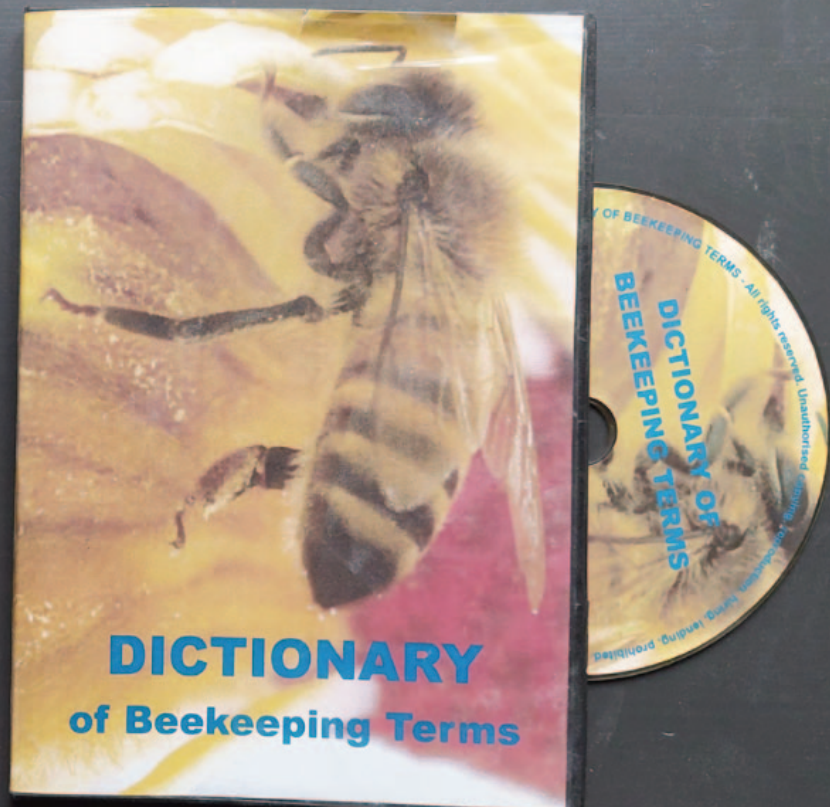
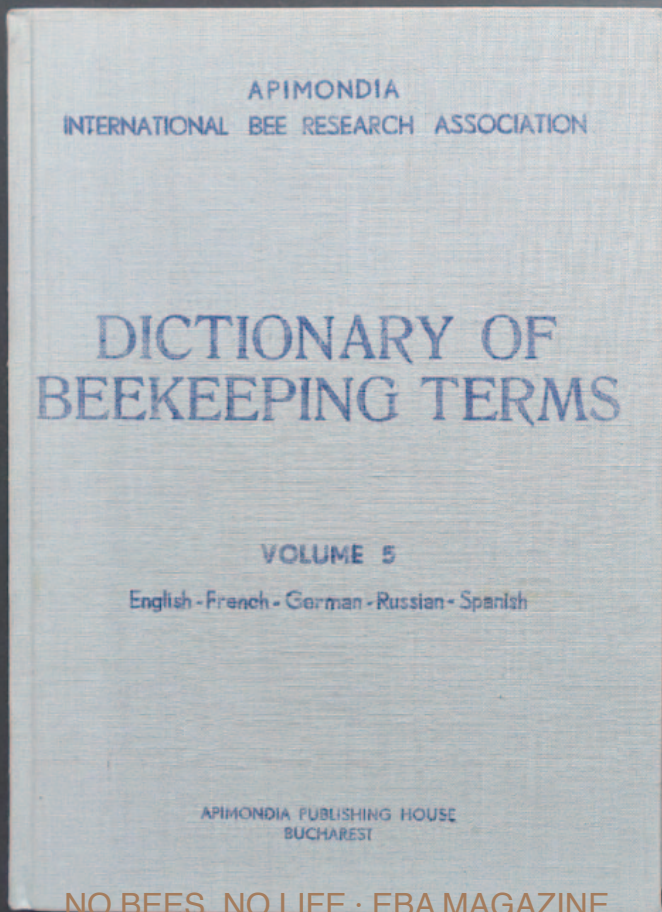
Furthermore it is important for scientists to better connect with beekeepers, as very much of their work, innovative ideas, experience and problems are of great relevance to them (not only for honey bee scientists, but also for the ones researching on beehive products, pollinators and environment). With WikiBeedia.eu (Figure 1), the BeeGuards consortium (introduced by Costa & Uzunov, Citation2024) created a platform that works as a community hub for beekeepers, honey bee scientists and everybody

else interested or involved in honey bees and beekeeping. Connecting scientists, beekeepers and relevant stakeholders in this joint effort, we



Figure 1. The WikiBeedia logo, inspired by the BeeGuards logo and branding style (see [www.beeguards.eu](http://www.beeguards.eu))

Figure 2. Previous generations of dictionaries of beekeeping terms. Left: Printed copy of "Dictionary of beekeeping terms" edited by Eva Crane and jointly published by Apimondia and International Bee Research Association in 1977. Other volumes included other languages, but English was always included and connected the terms to other languages even in other volumes. It contained more than 1,000 terms. The CD-Rom published by Apimondia in the 2000s provided a database of beekeeping terms in 24 languages



# European foulbrood

Page Discussion

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Translate this page

Other languages: [Deutsch](#) [English](#) [Gaeilge](#) [Malti](#) [Nederlands](#) [dansk](#) [eesti](#) [español](#) [français](#) [hrvatski](#) [italiano](#) [latviešu](#) [lietuvių](#) [magyar](#) [polski](#) [português](#) [română](#) [slovenčina](#) [slovenščina](#) [suomi](#) [svenska](#) [čeština](#) [Ελληνικά](#) [български](#) [македонски](#) [中文](#)

European foulbrood (EFB) is a damaging disease of honey bee larvae caused by the Gram-positive bacterium *Melissococcus plutonius*. The bacterium is fed to larvae in contaminated food, and whilst all larval stages are susceptible, only those that are infected early will succumb to the disease.<sup>[1]</sup> The bacterium replicates in the gut of the larvae until it fills the entire gut lumen,<sup>[2]</sup> and larval death usually occurs before capping. It remains unclear whether larval death is as a result of starvation due to nutrient competition with the bacterium<sup>[3]</sup>, or whether some pathogenic mechanisms may damage the peritrophic membrane<sup>[4]</sup>. Different strains of *M. plutonius* are known to vary in their ability to cause disease, a property known as virulence<sup>[5]</sup>. EFB was first described in 1885<sup>[6]</sup>, and is a statutory notifiable in some countries. Although called 'European' foulbrood, the disease has been reported from every continent where beekeeping is practiced. Larvae first show symptoms by twisting in their cells, no longer adopting the usual plump 'C' shape of healthy larvae. Next, the colour changes from the pearly white of healthy larvae, 'through to yellow passing on by degrees towards pale brown'<sup>[6]</sup>, and larvae lose segmentation as though melted down (Fig. 1). Irregularly placed scales can be observed in later disease stages.

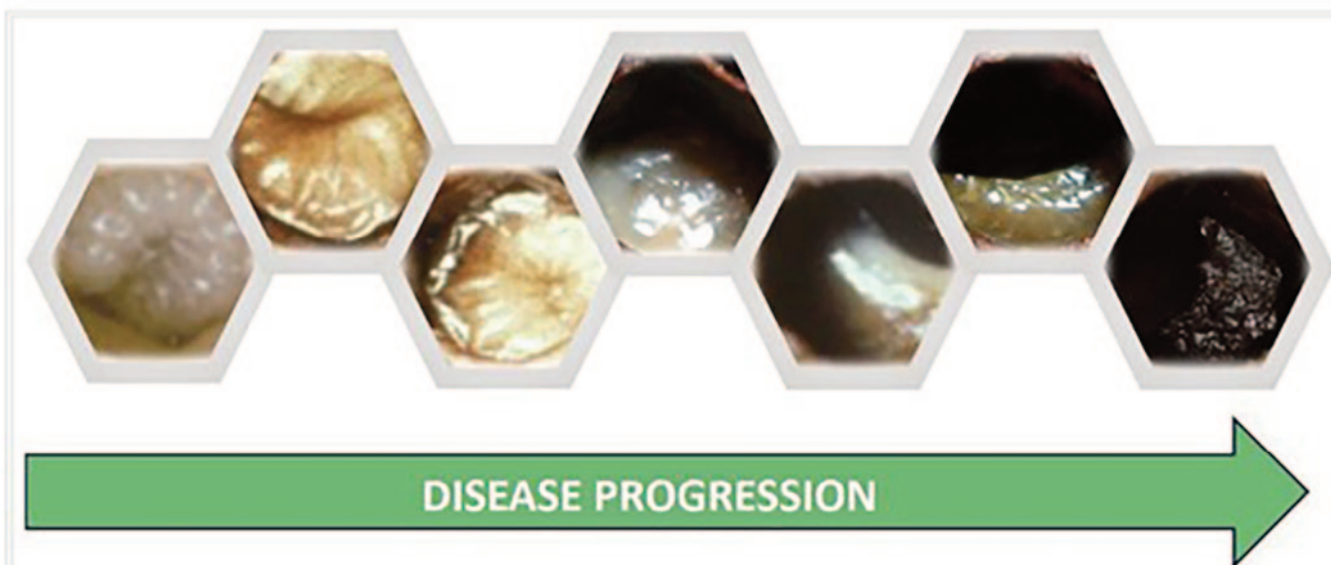


Fig. 1 Progression of European foulbrood in honey bee larvae from a healthy larva on the left. After infection larvae start to twist in their cells, before losing segmentation, and appearing melted down. Irregular scales can form later in the disease cycle<sup>[7]</sup>.

Figure 3. Screenshot of an exemplary article on “European foulbrood” containing a number of citations, a figure including caption, the language box on top of the article and different formattings, such as italics for species name or hyperlinked reference numbers, throughout the text

provide a tool so far unseen in the beekeeping sector.

Artificial intelligence has become more and more relevant as a source of information. Large language models, such as ChatGPT can even answer questions on beekeeping and therefore function as a beekeeping advisor. Morawetz et al. (Citation2024) showed that this works only to a certain degree. When the topic is very specific or complex, the answers become increasingly imprecise. In a follow-up paper more different chatbots were tested and a large variety of quality in

answers was found (Fabricius Kristiansen et al., Citation2024). Before we can fully rely on artificial intelligence we still are dependent on the knowledge and expertise of beekeepers, beekeeping advisors and scientists, therefore WikiBeedia can act as a platform for such a collaborative effort. According to the findings of Mansourian (Citation2024), beekeepers are keen to share their experiences through public online platforms and interact with beekeepers and beekeeping enthusiasts. They share a range of beekeeping tips and techniques, varying from hive management

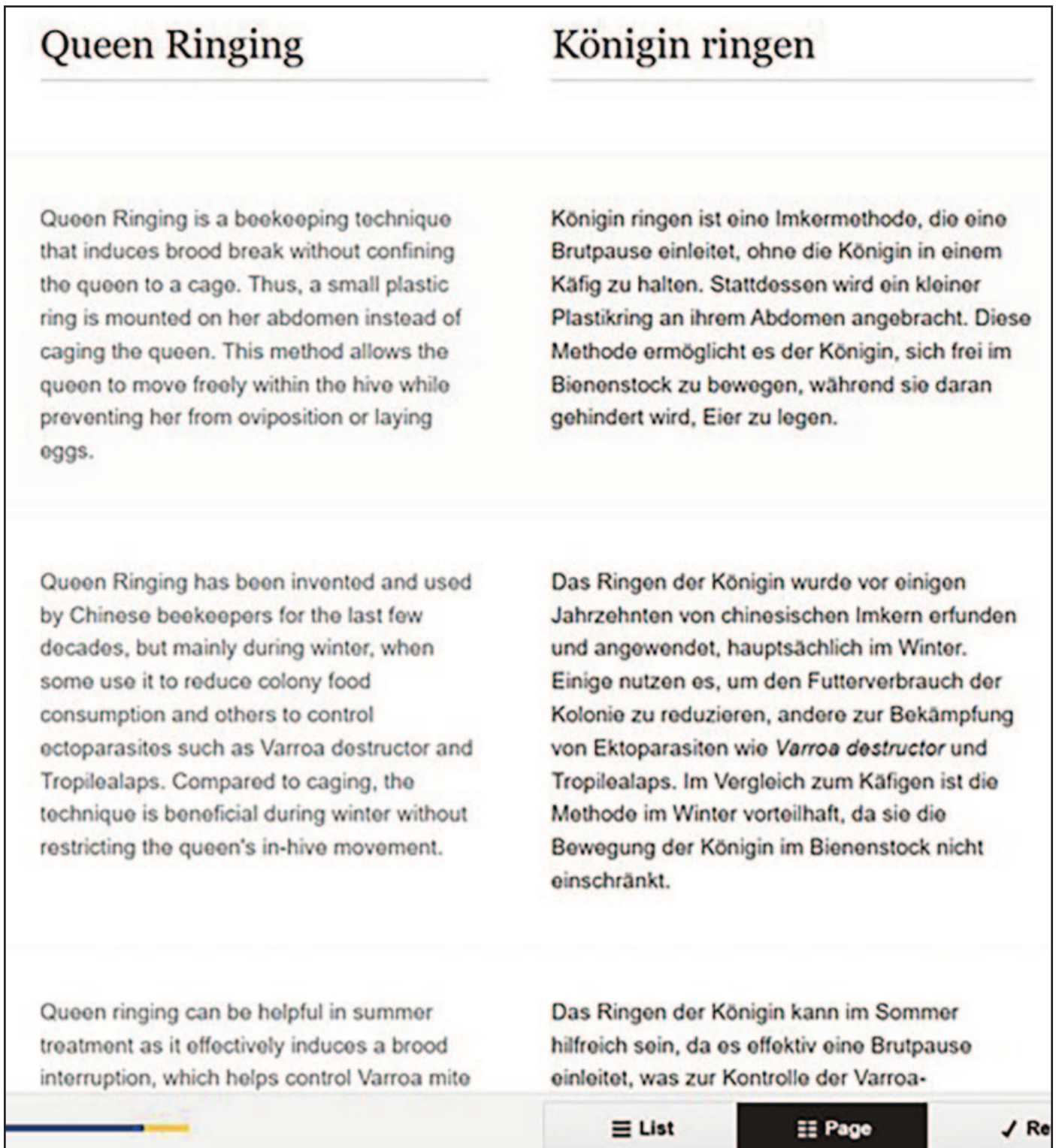


Figure 4. The translation window used in WikiBeedia as shown for the exemplary article of “Queen ringing.” The left column shows the original article in English. In the right column are the already translated paragraphs of the article in German. It is also possible to deviate from the translation, if local conditions are different from that of the template language

and honey production to seasonal hive management and bee feeding. In the ideal case, the curated knowledge collected in WikiBeedia would itself be used to train large language models and

make it more reliable in the field of beekeeping. We initially need the participation of not only our consortium but also regional and global beekeeping communities to fill our encyclopedia and to

translate as many articles as possible in all languages available.

area of the local language. You can imagine that hive management practices in Swedish would emphasize different issues than in Greek.

## Technical Implementation

For technical implementation we decided to use the wiki platform “miraheze.com” as it offers an uncomplicated approach to the customization of our encyclopedia and furthermore offers a very good support service. “Miraheze” uses Mediawiki, the same system which is used in the most common and widely known online encyclopedia Wikipedia. By using that platform we have almost no restrictions when it comes to editing. In our articles we can work with text formattings, videos, links, citations and everything else needed for presenting profound knowledge to the public. To make editing and translating of articles accessible to everyone we offer instructions directly on WikiBeedia.eu (<https://WikiBeedia.miraheze.org/wiki/Instructions>). Furthermore there are numerous other help-pages offered by miraheze, some of which are also linked at our instructions page. The only requirement one needs to get started contributing to WikiBeedia is an account, which can be created for free. The articles so far have been labelled with 6 different category tags: Research, Biology, Diseases, Hive management, Forage and Hive products.

## Multilinguistic Approach

Beekeeping dictionaries have been present in the last decades in various forms (Figure 2). We plan on making articles available in all European Union languages and furthermore Norwegian, Macedonian, Serbian and Chinese, but we are open to add further languages. For every article available in English we offer a “language box” (Figure 3), on top of the article containing the languages mentioned above. By clicking on one of these languages, one could switch between the article translations. If you click on a language with no translation, you can start translating yourself right away. Figure 4 shows the window which is used for translation. It is important to note, that instead of a word-by-word translation, which could – erroneously – be done by automatic translations, we prefer knowledgeable translations adapted to the conditions of the

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# Main Page

Main Page Discussion

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Other languages: Deutsch English Gaelige Malti Nederlands dansk eesti español français hrvatski italiano latviešu lietuvų magyar polski português română slovenčina slovenščina suomi svenska čeština Ελληνικά български 中文

## Welcome to WikiBEEdia!

Thank you for visiting WikiBEEdia! This encyclopedia on honey bee and beekeeping related topics was created within the EU project BeeGuards. It aims to be the world's number one encyclopedia on honey bees, beekeeping, honey bee diseases, hive management and research. It is available in many languages and beekeepers and honey bee researchers from around the globe are invited to contribute.

If you have any questions, please contact us at info@wikibeedia.eu. Some instructions for writing, editing and translating articles can be found here, if you want to contribute, please use the following page as a starting point: Instructions



## Categories

WikiBEEdia is organised in different categories to make it easier to navigate. So far we divided our articles in six categories:

- Research
- Biology
- Diseases

# How to replace a queen

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### Beginning

SAFE ADDITION OF QUEEN

ACQUISITION

Adding paired or unpaired queens

Outside the drone period (only mated queens)

Adding a queen cell

DID YOU KNOW

Other languages: English dansk

## SAFE ADDITION OF QUEEN

The queen - the centre of the bee family "For a bee family to function optimally, it needs a young queen, maximum two years old. The queen's characteristics determine how the entire bee family functions. Read this text and prepare yourself on how best to add your new queens. It can save you a lot of aggravation and money."

## ACQUISITION

Queens can be acquired in several ways. You can buy unpaired or paired queens. A more recent phenomenon is to add creep-ready queen cells. We recommend that you buy queens from queen breeders who participate in the Danish Beekeepers' Association's



# JÜRGEN BINDER CANDIDATE FOR THE POSITION OF VICE – PRESIDENT OF APIMONDIA



Jürgen Binder is a professional beekeeper and a member of our association Deutscher Berufs- und Erwerbsimkerbund e.V. (German Professional and Commercial Beekeepers' Association).

In addition to his beekeeping business he founded the Prof. Ludwig Armbruster Beekeeping School in 2014. He has been training new beekeepers for over 10 years and teaches recreational and professional beekeepers the

latest operational, scientific and ecological developments at an international level.

For decades, Jürgen Binder has not only campaigned for improved beekeeper training, but has also continuously called for a stronger focus on more ecological beekeeping and agriculture with stricter pesticide control through information events and campaigns. He also demonstrated his great commitment to the beekeeping industry by

founding the New Beekeepers' Association in 2022, which he chairs as President and which further expands his room for manoeuvre. In this context, he was recently able to introduce a group certification for organic-beekeeping, which was previously not possible in Germany. This makes it affordable also for many small beekeeping businesses to switch to organic beekeeping.

Jürgen Binder has been campaigning for the organisation of the Apimondia in Germany for many years. He is convinced that this world beekeeping congress and trade fair has an important impact in promoting international relations between beekeepers, the global transfer of knowledge, the ecological orientation of beekeeping and agriculture and ultimately a healthier, pesticide-free environment for the benefit of humans and animals. We are

convinced that Jürgen Binder, as Vice President of Apimondia, can make a critical contribution to advancing these important goals for us beekeepers internationally. We therefore support his candidature for this position.

**Annette Seehaus - Arnold**  
President

(text source- Letter of recommendation – from German Professional and Commercial Beekeepers Association (DBIB))



# BEE THEME PARK AND BEE PARADISE IN ANDRAŽ NAD POLZELO

On Sunday, June 1, 2025, the Municipality of Polzela, together with its partners, ceremonially opened the Bee Theme Park and Bee Paradise on Jelovski hrib (Jelovski hill) in Andraž nad Polzelo, near the famous Jelovska Linden Tree and Kastelka's landmark.

This is more than just a new spatial arrangement – the project represents an important step toward education, awareness-raising, and the

preservation of natural and cultural heritage. The project is headed by the Municipality of Polzela and it has been successfully submitted to the EAFRD public call for proposals with the support of the European Union and the Republic of Slovenia, securing 80 percent of the funding as non-repayable grants.

The initiative to establish the park came from local residents Ivica and Konrad Brunšek, who



generously donated part of their land. Their idea was embraced by Mayor Jože Kužnik, and the concept quickly evolved into concrete action. The municipality invited three important partners to collaborate: the Brunšek – Krk Farm, beekeeper Marko Golob, and Polzela Elementary School.

On the slopes of Jelovski hrib (Jelovski hill), a honey orchard was created with more than 200 melliferous plants, marked with informational boards and QR codes.

The theme park, named Bee Paradise, offers visitors the opportunity for in-depth learning about bees, plants, and their role in the ecosystem.

All beekeeping associations in Slovenia are also included in a special way, as they are the ones to whom each planted melliferous symbolic tree – from lindens to cherries and chestnuts – is dedicated.

The orchard features a beautifully designed walking path that provides visitors with a pleasant and educational experience of nature.

The project also includes a traditional Slovenian apiary, equipped with beehive panels that were decorated with ideas by pupils of Polzela

Elementary School. Inside the apiary, visitors can learn about beekeeping products and learn about apitherapy. This experience is curated by long-time beekeeper Marko Golob.

The next educational component holds special significance as well, with the Polzela Elementary School having prepared worksheets and outlines for school group visits, thus ensuring the educational value of the project. This way, our children will learn about the importance of bees and responsible environmental stewardship from an early age.

The ceremony on June 1st was attended by representatives of municipalities, beekeeping associations, and public institutions, and was honored by the presence of Boštjan Noč, President of the Slovenian Beekeepers' Association and President of the European Beekeepers' Association.

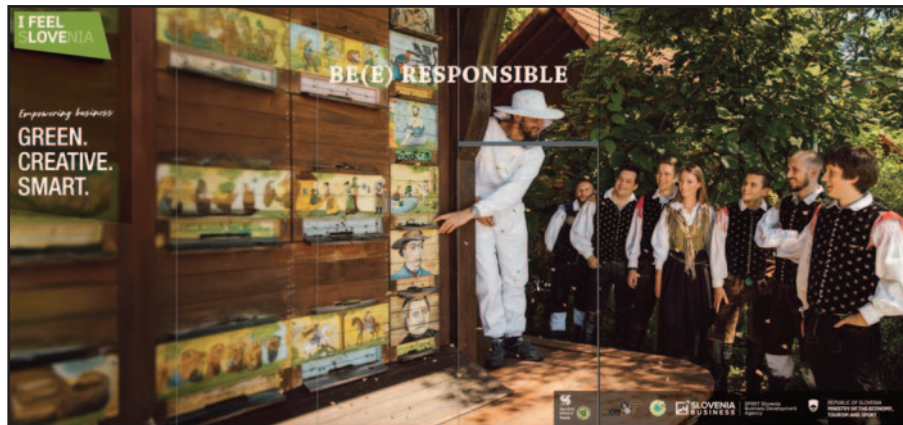
With this project, the Municipality of Polzela continues its vision of sustainable development, fosters the preservation of natural heritage, and boosts tourist visibility of local environment.

# SLOVENIA AT APIMONDIA IN DENMARK

Slovenian beekeeping will be presented at Apimondia in Denmark in September, the overall look of the stand has been created!

Thanks to the Slovenian Beekeeping Academy and our country Slovenia, who provided us with the finances to enable a joint presentation of 12 business entities from the field of beekeeping!

Find our Slovenian exhibition space, we look forward to your visit!



# VISIT THE CARNIOLAN BEE HOUSE IN SLOVENIA

Are you looking to offer your guests something truly unique and deeply local in Slovenia? Discover the Carniolan Bee House, located in the charming town of Višnja Gora, Slovenia. We believe that together, we can offer your clients an authentic Slovenian experience your guests will remember – with honey tasting, cultural stories, and a stay they'll rave about. We would be delighted if you consider including the Bee House in your tours, excursions, and travel itineraries. What we offer at Carniolan Bee House:

- Unique accommodation in wooden honeycomb-shaped rooms
- Interactive exhibition about beekeeping and Carniolan bee, including a guided honey tasting
- Local honey delicacies
- A tourist info center featuring a range of authentic local products

Additional benefits for your guests:

- Located just 25 km from Ljubljana, near highway
- Parking for cars and buses
- Perfect starting point for exploring Slovenia
- We serve a traditional Slovenian breakfast made from local produce
- Situated in the historic medieval town center of Višnja Gora
- Access to nearby cycling and hiking trails
- Offers an authentic connection with local heritage and nature

We'd be happy to tailor the visit experience to your customer's needs and interests. Please don't hesitate to get in touch with us for any additional information, visit coordination, or partnership opportunities.

Website:

<https://www.hisakranjskecebele.si/en>



# The Carniolan Bee House



A most special house, called the Carniolan Bee House, has opened its doors in Višnja Gora. With it, Slovenia wants to revive the unique beekeeping story of one of Slovenia's most important beekeeping families, the Rothschild, which is responsible for the present-day spread and visibility of the Carniolan bee in the world. The Carniolan bee is an *apis mellifera* subspecies which is the second most common honeybee subspecies in beekeeping. Members of the Rothschild family introduced the Carniolan bee to the world more than 150 years ago. Today, they tell the story of beekeeping, the interesting biology of the Carniolan bee, and, above all, they teach visitors from Slovenia and abroad about the important impact bees have on the environment and on people. The Carniolan Bee House is home to the Tourist Infor-

mation Centre, where guests can learn all about what the house and the surrounding area have to offer. The café with a summer garden is a space with a selected range of honey products and products from local suppliers.

The ApiLab Centre of Innovative Technologies is dedicated to improving the competencies of small and medium-sized companies and provides various training courses.

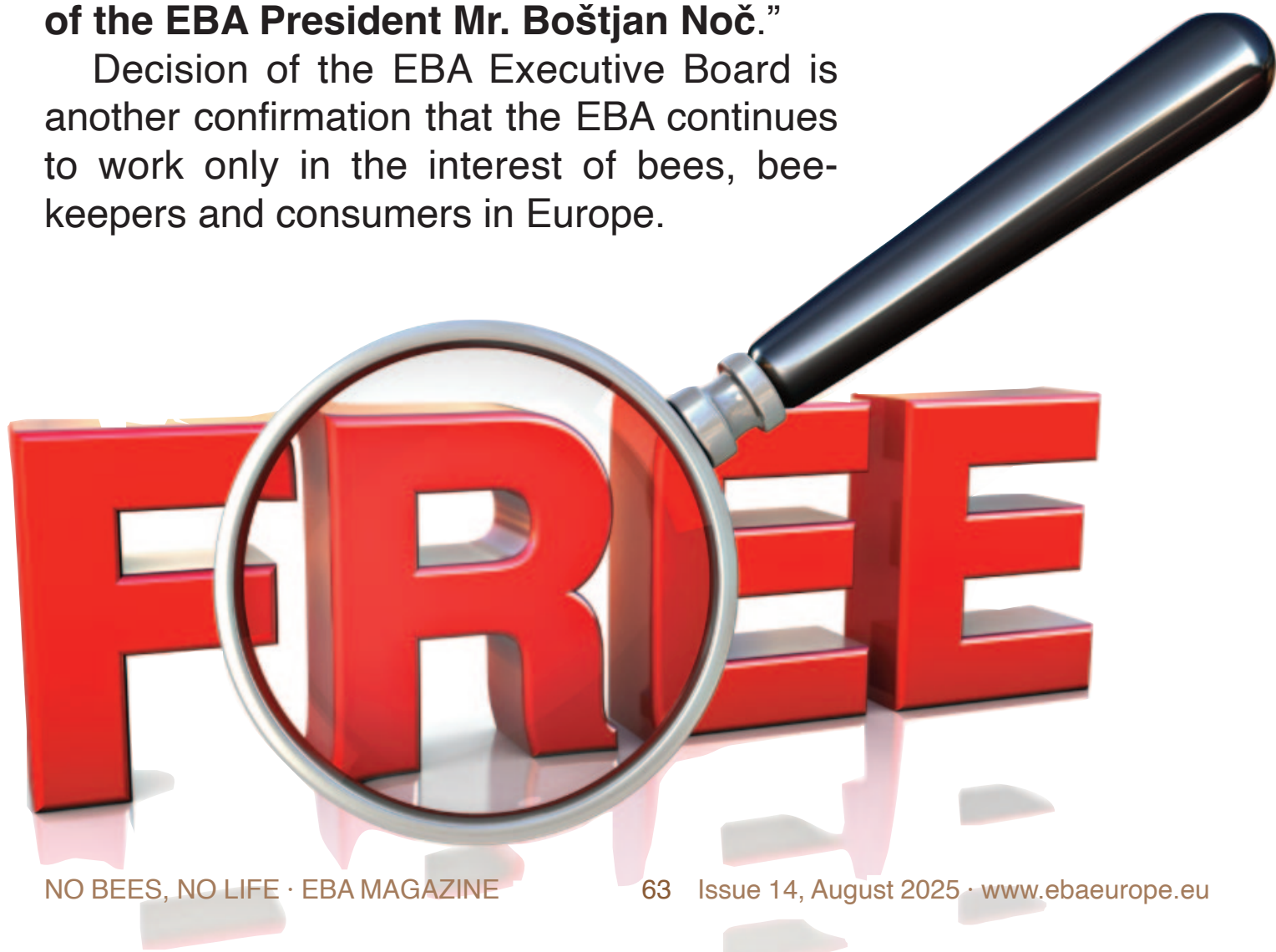
The centrepiece of the Carniolan Bee House is the Carniolan Bee Exhibition, which is arranged circularly in four rooms and teaches visitors about the bee; it also features a live beehive.

The Carniolan Bee House brings together bees, people, and beekeepers under one roof.

# TO THE EBA WITHOUT MEMBERSHIP FEE

At the meeting of the EBA Executive Board, on the proposal of the EBA President Mr. Boštjan Noč, an important decision was made regarding membership in the EBA in the upcoming period: **“Membership in the EBA is free for the duration of the mandate of the EBA President Mr. Boštjan Noč.”**

Decision of the EBA Executive Board is another confirmation that the EBA continues to work only in the interest of bees, beekeepers and consumers in Europe.



# SPONSORSHIP REQUEST

## AND METHOD OF ADVERTISING IN THE MAGAZINE

On behalf of the European Beekeeping Association (EBA), I am writing to seek your support in the form of sponsorship to help ensure the smooth and effective operation of our Association.

The EBA is dedicated to promoting and supporting beekeeping across Europe. The Association was founded out of necessity, as bees and beekeepers are essential for our ecosystem and society. Without beekeepers there are no bees, and without bees there is no pollination, leading to a lack of food on planet Earth.

EBA works for bees, beekeepers and consumers.

Our mission is to:

1. Fight against counterfeit honey that flooded the European market;
2. Introduction of incentives per beehive as agro-ecological programme;
3. Fight against the improper use of chemicals that are harmful to bees;

In return for your generous support, we offer various sponsorship benefits. We believe that this partnership would be mutually beneficial and would significantly contribute to the advancement of the European beekeeping sector.

### ADVERTISING IN THE MAGAZINE:

1. Through sponsorship packages;
2. It is possible to pay for an ad only for 1/4 page (100 euros), for a larger area by agreement. The entire page cannot be obtained, it belongs only to the General Sponsor.

IT CONTINUES 





# EBA

## sponsorship packages

### **GOLD sponsor - 5.000 euros:**

Advertisement on the EBA website  
Presentation at all EBA events, logo on all EBA correspondence  
12 advertisements in the EBA monthly e-magazine in A4 page size

### **SILVER sponsor - 3.000 euros:**

Advertisement on the EBA website  
Presentation at all EBA events, logo on all EBA correspondence  
12 advertisements in the EBA monthly e-magazine in half A4 page size

### **BRONZE sponsor - 2.000 euros:**

Advertisement on the EBA website  
12 advertisements in the EBA monthly e-magazine in the size of 1/4 A4 page

### **EBA SUPPORTER - 1.000 euros:**

Advertisement on the EBA website  
12 advertisements in the EBA monthly e-magazine in the size of 1/8 A4 page

These are basic packages, but we are open to different forms of cooperation, which we agree on individually. We would be delighted to discuss this opportunity further and explore how we can align our goals with your organization's values.

Thank you for considering our request. We look forward to the possibility of working together.

Yours sincerely,

**Boštjan Noč**  
President of the European Beekeeping Association

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The contents of the texts and advertisements are the responsibility of the authors.

The responsibility for the correctness of the English language in the magazine lies with the authors of the texts.

The editor reserves the right to publish a larger advertisement than the size specified by the sponsorship package, if it improves the design of the magazine.

**Advertising in the magazine:** 1. Through sponsorship packages; 2. It is possible to pay for an ad only for 1/4 page (100 euros), for a larger area by agreement. The entire page cannot be obtained, it belongs only to the General Sponsor.

The total number of pages in the magazine is not fixed.

There are no fees for published texts and photos.

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