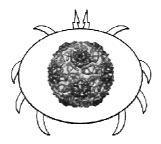




COLOSS Workshop "Varroa and Viruses"



Organized by the Swiss Bee Research Institute Bern, Switzerland 1st to 4th of November 2010

Dear colleagues

It is our pleasure to welcome you to the international Work Shop on Varroa and Viruses organised by the Swiss Bee Research Centre, in Magglingen near Biel, Switzerland.

We are grateful to all participants for their contributions and are looking forward to the stimulating discussions on gaps, challenges and perspectives in varroa and viruses research.

Financial support is granted by COST via the Action FA0803 COLOSS.

The Local Organising Committee for the Work Shop

Vincent Dietemann, Jochen Pflugfelder, Peter Neumann Bern, Switzerland, Wednesday, 06 October 2010

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Agenda

Monday 1.11. 2010	
15:00 – 19:00	Arrival, registration and informal social gathering
19:00	Apero and Dinner

Tuesday 2.11. 2010	
08:30 - 09:00	Registration
09:00 – 09:15	Welcome & Greetings by Brigitta Gadient
09:15 – 09:30	Scientific agenda and organizational matters
09:30 – 10:45	Wolfgang Nentwig: biological invasions
10:45 – 11:00	Discussion
11:00 – 11:30	Energy Break
11:30 – 12:00	Dennis Anderson: Diversity, Distribution and Host-Specificity of Varroa Mites
12:00 – 13:00	Discussion
13:00 – 14:00	Lunch
14:00 – 14:30	Debora Smith: Co-evolution between <i>Varroa</i> and <i>Apis cerana</i> populations: does it exist, and can we make use of it?
14:30 – 15:30	Discussion
15:30 – 16:00	Energy Break
16:00 – 16:30	Peter Rosenkranz: Varroa reproduction in honey bee colonies: Interactions between host and parasite and possibilities for a biological control.
16:30 – 17:30	Discussion
20:00 – open	Social dinner

Wednesday 3.11.2010	
09:00 - 9:30	Ingemar Fries: Mechanisms of varroa tolerance (provisional title)
09:30 – 10:30	Discussion
10:30 – 11:00	Energy Break
11:00 – 11:30	Stefan Fuchs: Population processes of hosts and parasites in the Varroa - honeybee system
11:30 – 12:30	Discussion
12:30 – 13:30	Lunch
13:30 – 14:00	Keith Delaplane: Lessons learned in the fight against Varroa
14:00 – 15:00	Discussion
15:00 – 15:30	Energy Break
15:30 – 16:00	Diana Sammataro: Diagnosing Varroasis
16:00 – 17:00	Discussion
17:00 – 18:00	Bee Book method standardization
18:00 – open	Social dinner

Thursday 4.11. 2010	
08:30 – 09:00	Nor Chejanovsky: Honey bee viruses: dormant and transmissible phantoms
09:00 – 10:00	Discussion
10:00 – 10:30	Energy Break
10:30 – 11:00	Joachim de Miranda: virus diagnosis and transmission (provisional title)
11:00 – 12:00	Discussion
12:00 – 13:00	Bee Book method standardization
13:00 – 14:00	Lunch
14:00 - open	Optional sightseeing in Bern
Departure	

CONFERENCE LOCATION

Bundesamt für Sport BASPO

Hauptstrasse 247CH-2532 Magglingen

Tel +41 32 327 61 11

Fax +41 32 327 64 04

http://www.baspo.admin.ch/internet/baspo/de/home/das_baspo/kontakt.html

CONTACT FOR FURTHER INFORMATION

Vincent Dietemann, Jochen Pflugfelder, Peter Neumann

E-mail: vincent.dietemann@alp.admin.ch

jochen.pflugfelder@alp.admin.ch

peter.neumann@alp.admin.ch

Phone: +41 31 323 82 048, Fax: +41 31 323 82 27, www.apis.admin.ch

Diversity, Distribution and Host-Specificity of Varroa Mites

Anderson Denis

CSIRO Ecosystem Sciences Canberra, Australia

Author for correspondence: E-mail: Denis.Anderson@csiro.au

Phone: 61-2-62464148

This presentation summarizes some important insights into the genetic diversity, geographical distribution and host-specificity of *Varroa* mites, gained from studying DNA variation among different mite populations. Gaps in current knowledge and challenges and perspectives for future research will be highlighted and discussed.

The genus *Varroa* currently consists of 4 well-defined species, *Varroa* destructor, *V. jacobsoni*, *V. underwoodi* and *V. rindereri*. Further studies on *Varroa* genotypes in the Philippines and Sri Lanka may well reveal more species.

Studies of mtDNA cox1 gene sequence variation among *V. destructor* and *V. jacobsoni* on *Apis cerana* (the native bee host) throughout Asia have identified 9 and 15 large populations of genetically similar mites within each species respectively. These populations have been referred to as 'haplogroups'. *A. cerana* that inhabit small Asian islands (e.g. Java, Bali and Flores Islands in Indonesia) host mites of just 1 haplogroup, whereas several haplogroups have been identified on *A. cerana* on mainland Asia, 3 on *A. cerana* in China alone. Unpublished data indicates that the biogeography of different *A. cerana* populations and mite haplogroups is similar.

Studies on other mtDNA gene sequences (cox3, atp6 and cytb genes) of V. destructor and V. jacobsoni infesting A. cerana in north-east Asia have revealed numerous 'haplotypes' within the haplogroups of each species.

Until 2008, mites of just two haplogroups of *V. destructor* (Korea 1 (K1) and Japan 1 (J1)) were known to have switched-host and colonized *A. mellifera*. Mites of each haplogroup showed almost no microsatellite or haplotype polymorphism on *A. mellifera*, indicating that they each resulted from two independent host-switches (from *A. cerana*), during which there were severe genetic bottlenecks. These two haplotypes are now recognized as the first haplotypes of their respective haplogroup (K1-1 and J1-1 respectively).

In 2008, mites of a J1 (Java) haplogroup of *V. jacobsoni* were found to have switched-host and colonized *A. mellifera* in Papua New Guinea, but not in other countries (Indonesia or the Solomon Islands). In 2010 a further 2 haplotypes of *V. destructor* (K1-2 and J1-5) were found to have switched-host and colonized *A. mellifera* in north-east Asia. Interestingly, no haplotype that has colonized *A. mellifera* has yet been found on *A. cerana*.

Data collected to date indicates that the majority of *Varroa* haplotypes have not colonized *A. mellifera*, simply because they lack the ability to lay eggs and produce offspring on *A. mellifera* brood.

Honey bee viruses: dormant and transmissible phantoms

Chejanovsky Nor

Entomology Department, The Volcani Center, Bet Dagan, Israel

Author for correspondence: E-mail: ninar@volcani.agri.gov.il

Honey bees are exposed to numerous pathogens including viruses, bacteria, fungi and parasites. Viral infections were associated with beehive decline. Two types of viral-induced pathologies were mainly linked with substantial colony losses worldwide: paralysis and deformed wing syndrome. The most important viruses frequently associated with paralysis were the Acute bee paralysis virus (ABPV), the Kashemir bee virus (KBV), the Israeli acute paralysis virus (IAPV) and the Chronic bee paralysis virus (CBPV), while the Deformed wing virus (DWV) and the Varroa destructor virus-1 (VaDV-1) were associated with the deformed wing syndrome. Classical approaches including different type of bioassays, immunological, virological and cell biology techniques were recruited to learn the mechanisms underlying the above viral infections. The picture that emerges from the above studies indicates that for most of the cases the above viruses are present as dormant (covert) infections that become activated upon exposure of the colonies to still undefined stress factors. While for some of the above viruses *Varroa destructor* serves as a vector and an elicitor of infection, for others the situation is less clear. The recent introduction of molecular techniques like qualitative and quantitative RT-PCR enabled further progress in understanding the nature and basis of the viral-induced pathologies, however, the lack of well controlled in vivo and in vitro experimental models are still hampering further progress. The present limitations, gaps and prospective for further advancing our knowledge on viral infections of the honey bee will be discussed.

Methods of honey bee virus diagnosis and virus transmission pathways: Gaps, challenges and perspectives

De Miranda Joachim

Swedish University of Agricultural Sciences Department of Ecology, Uppsala, Sweden

* Author for correspondence: E-mail: joachim.de.miranda@ekol.slu.se

Honey bee viruses have become a major consideration in bee health management during the last 30 years, in large part due to the active transmission of several of these by the exotic ectoparasite Varroa destructor, but increasingly also due to their opportunistic association with other environmental stresses, both natural and manmade. This enhanced profile of viruses in bee health has coincided fortuitously with the revolution in molecular biology and biotechnology, which has greatly increased the sensitivity of pathogen detection and the molecular information available for both host and pathogen, and more recently also with an increased social and political awareness of the importance of honey bees to agriculture and food production. These three developments have brought into focus the large gaps in our understanding of the interactions between honey bees and their viruses, the conflicts and inadequacies in the methods of pathogen detection and the conclusions drawn from them, the need to develop our knowledge into practical solutions and our responsibility to society for the accuracy and validity of the information we provide. This presentation will address a number of these issues and provide some perspective on how to overcome the deficiencies in our knowledge, resolve the conflicts in our techniques and increase the accuracy of our information flow.

Lessons learned in the fight against Varroa

Delaplane Keith S.

University of Georgia, Athens, GA, USA

Author for correspondence: E-mail: ksd@uga.edu

The non-natural parasitic relationship of the mite *Varroa destructor* on the Western honey bee Apis mellifera has become a benchmark in bee science and culture on par with Langstroth's moveable frame hive. In those regions affected by Varroa, beekeeping history within living memory can be divided into halves - a time pre-Varroa when honey bees were self-sustaining, and a time post-Varroa when honey bees die in the absence of ceaseless inputs by the beekeeper. In the latest episodes of bee decline it is generally agreed that morbidity is a result of interacting factors, not the product of any one including Varroa. Nevertheless, it is a premise of this workshop that Varroa is at the center of the problem and perhaps receiving less attention than it's due. It is my opinion that Varroa, more than anything else, has morphed beekeeping from an agricultural industry inherently chemical-averse to an industry exceptional in its chemo-centricity. The speed of this transformation is dizzying and its irony huge - given that today we accept as normal the use of synthetic acaricides inside hives of living honey bees. The introduction of pesticides into beekeeping has meant the introduction of problems once the worry of other sectors of agriculture - pest chemical resistance and non-target chemical effects. Today it is understood that in-hive chemicals are not the answer to sustainable honey bee health maintenance; rather, they may be part of the problem. Progress toward non-chemical solutions has been mixed: cultural control is impractical for all but the smallest beekeepers, and specialized genetic host resistance has proven difficult to identify, propagate, and deliver at the industry scale. I propose that the time is right to revisit the problem at its most basic levels - what are the evolutionarily-driven strategies employed by social bees to withstand pests and predators? Recent work in the context of superorganism theory hints at the adaptive benefits of multiple mating that increases intra-colony genetic heterogeneity and phenotypic robustness against many types of colony threat. Epidemiological theory points to host density as a regulator of parasite virulence. These are two of many areas of fundamental biology ripe for study and application in the fight against Varroa.

Assessment of varroa mite tolerance in honey bee colonies

Fries Ingemar

Swedish University of Agricultural Sciences Department of Ecology, Uppsala, Sweden

Author for correspondence: E-mail: ingemar.fries@ekol.slu.se Phone: +46-18-672073

By estimating the mite population size between two dates with free reproduction of the mites a growth rate can be obtained that is comparable between different colonies, regardless of infestation level and at least in part independent of the number of days the measurements include. With this information the growth rate can be calculated from

I.
$$\chi = e^{r * d}$$

where

 χ = the number of multiples by which the population has grown

e = the natural logarithm

r = growth rate per day

d = number of days during which the measurement occurred

Example: The measurement took place during 65 days (d = 65). Mite population is estimated to have increased from 100 to 580 (= 5.8). Formula I can now be written as II. $r = ln\chi/d$

hence
$$r = ln (5.8) / 65 = 0.027$$

Thus a growth rate of 2.7% per day in this case. This measurement should provide a basis for assessing the varroa tolerance. Options for measurement of the population growth will be discussed.

Population processes of hosts and parasites in the Varroa - honeybee system.

Fuchs Stefan

Institut für Bienenkunde Oberursel, Karl-von-Frisch-Weg 2, Oberursel, Germany

Author for correspondence: E-mail: s.fuchs@bio.uni-frankfurt.de.

Phone: ++49 6171 21278

Damages to honeybee colonies are closely linked to *Varroa destructor* population growth, which in turn depends on colony development and brood cycle. These relationships are well understood and a considerable number of studies have integrated the reproductive biology and life history of the mites into consistent within-colony population models. However, the dynamics of mite population development in populations of honeybee colonies involving colony fission, colony death and mite transfer between colonies by drifting and robbing are far less well understood.

A preliminary attempt has been undertaken some time ago to model these processes using multi-agent simulations. Three successive versions of this model incorporating increasing complexity still fell short of a concise finalization due to limitations in human and computing resources, but still showed the potential for a broader understanding of the varroosis epidemiology in populations of honeybee colonies which are encourage further development of this approach.

Even in at the current preliminary state the models yielded interesting insights. As major trends, they stressed the crucial role of the substantial mite transfer by robbing and drifting between colonies during colony breakdown for bee population decline, thus emphasizing the role of colony spacing. They also showed that reduction of colony infestation during swarming could counteract population decline. In particular, less obvious factors as colony longevity emerged as possibly essential factors. As a main result the models indicated possible conditions where complete bee population eradication may not a likely long-term outcome of Varroa parasitation, leading to stable parasite – host equilibria. To understand more precisely these conditions may help to reflect the impact of current beekeeping practices on mite infestation, and may help to devise colony management methods which exploit these insights to reduce the impact of varroosis on beekeeping.

Breeding for Varroa-Resistant Honey Bees in the United States

Harris Jeffrey 1*, Rinderer Tom E. 1, Spivak Marla 2

¹USDA, ARS Honey Bee Breeding, Genetics & Physiology Laboratory Baton Rouge, USA ²Department of Entomology, University of Minnesota, USA

* Author for correspondence: E-mail: Jeffrey.Harris@ars.usda.gov

Phone: 225-767-9284

Three programs in the U.S. that are focused on breeding varroa-resistant honey bees will be discussed. These include the breeding of (1) the Minnesota Hygienic stock, (2) the Russian stock and (3) bees that carry the Varroa Sensitive Hygiene trait. Programs will be compared and contrasted as to general breeding philosophy, levels of varroa resistance, levels of other economically important characteristics, technology transfer and delivery of material to the beekeeping industry, and sustainability in the near future. Hurdles to complete transfer of breeding to commercial interests will be outlined, and some suggestions for improvement will be highlighted. New methods for selection of varroa resistance will be discussed, especially with regard to the potential of marker-assisted selection to replace field selection of breeding parents. Current efforts in development of markers for the Varroa Sensitive Hygiene trait will be featured. Finally, discussion of novel breeding approaches and/or the selective breeding for new (or under appreciated) varroa resistance mechanisms will be solicited from conference participants.

Varroa destructor influences behaviour of honey bee foragers Apis mellifera

Kralj Jasna¹*, Fuchs Stefan ²

¹National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia ²Institut für Bienenkunde (Polytechnische Gesellschaft), Goethe-Universität Frankfurt am Main, Oberursel 61440, Germany

* Author for correspondence: E-mail: jasna.kralj@nib.si,

Phone: +386 59232773

Varroa destructor, a major pest of honey bees, alters flight behaviour of forager honey bees to the effect that they might not return to the colony. This explains the observation that a higher proportion of foragers leaving the colony are infested than of those returning to the colony. Behavioural experiments showed that infested workers had prolonged flights and impaired orientation. In returning experiments of foragers released at some distance from the colony infested foragers take longer time to return or do not return at all. Prolonged flights were also confirmed by using radio frequency identified (RFID) workers registered at the entrance for their departure and return. This method also confirmed that infested workers vanished from the colony earlier indicating loss of the parasite by non-returning and/or shorter life span of infested bees. In a visual orientation test infested bees scored lower indicating that orientation to the nest entrance is impaired by deficiencies in sensory and/or neural processing. A neuronal basis for behavioural deficiencies was supported by investigating learning abilities of infested foragers which was tested using the proboscis extension reflex (PER). These experiments revealed differences in non-associative learning although bees were equally responsive to sugar concentrations. Infested foragers showed a decrease in proboscis extension response in sensitization, habituation and 1 min olfactory conditioning test. Similar effects on foragers' behaviour were demonstrated in bees infected by Nosema sp. indicating that the altered behaviour of foragers by V. destructor is a general response serving as a defence mechanism to remove parasites or pathogens from the colony.

Varroa mite control: successes, failures, side effects and challenges

Pettis Jeff

USDA-ARS Bee Research Laboratory, Beltsville, MD 20705

Author for correspondence: E-mail: Jeff.pettis@ars.usda.gov

Phone: +301 504-7299

The parasitic mite Varroa destructor has changed the way honey bees (Apis mellifera) are managed worldwide. Since the host shift from Apis cerana, Varroa has made beekeeping more difficult and the search continues for the perfect miteresistant bee stock or highly effective and yet non-toxic control agent. Varroa control efforts can be separated into three broad areas, resistant bee stock selection, chemical and non-chemical control compounds and cultural controls. The use of these stragegies in combination or alone have still not resulted in the elimination Varroa as a major factor in colony health. In many areas of the world mite-resistant stocks have demonstrated that they can tolerate mite populations but these stocks have not found wide acceptance by beekeepers. Most synthetic chemicals are added to slow release strips and the bees pick up the chemical and through contact the mites are killed; examples include fluvalinate and coumaphos. Although these controls have been highly successful in the short run they result in residues in wax and mites have become resistant to many compounds. Of the non-chemical additives items such as powdered sugar to dislodge mites and spores of fungi to kill Varroa have been tried with limited success. Many promising control agents in laboratory screening, have then failed in whole hive experiments as distribution within the hive can be a major obstacle. Several cultural controls are effective in slowing mite growth; these include making colony divisions, the removal or drone brood and screen bottom boards. Future control efforts might include self destrucing strips that would reduce exposure time and thus slow the buildup of resistance and the continued selection and use of mite resistant stock. Other promising areas are the use of RNAi to target metabolic pathways in Varroa that would result in varroa control. Virus transmission by Varroa continues to heighten the impact of the mite alone. We should strive to find ways to help colonies deal with Varroa parasitism; surely the widespread use of miticides keeps susceptible stock alive instead of letting it die out and the more tolerant bee stocks survive. If we understood Varroa biology better and how the mite limits its reproduction in Apis cerana then perhaps we could find ways to select these characteristics or behaviors into European bees. Until we better understand Varroa and its association with its natural host, we are sure to continue to suffer from the impact of the parasite on European bees.

Challenges for developing biopesticides against varroa

Meikle William G. 1*, Mercadier Guy 2

¹Honey Bee Research Unit, Kika de la Garza Subtropical Agricultural Research Center, USDA-ARS, Weslaco, USA; ²European Biological Control Laboratory, USDA-ARS, Montferrier-sur-Lez, France

* Author for correspondence: E-mail: william.meikle@ars.usda.gov Phone: 956-969-5019 (office), 956-969-5033 (fax), 956-463-8293 (cell)

Effective control of varroa mites using biological control agents, such as entomopathogenic fungi, would reduce the number and amount of chemical agents introduced in bee hives, beneficial for both bee and human health, and would likely retard or eliminate the development of resistance by varroa. Several research groups have identified and tested candidate fungi. With multiple applications of the fungus *Beauveria bassiana* we observed significantly higher varroa mite fall in treated hives compared to control during the application period, and significantly lower mite fall starting 10 d after application. However, we did not observe significantly lower phoretic mite densities between treated and control, and other research groups have reported mixed success, albeit usually with single applications and with a different fungal species. Several questions concerning the varroa/ fungus/ honey bee interaction need to be addressed, including:

- 1) To what extent are bees affected by the fungus? We did not observe large losses of adult bees after application, but in that case how do adult bees protect themselves from the fungus? Is it due to grooming, or to fungistatic compounds in their cuticle, or to other factors? Even multiple applications did not appear to affect brood, so how is brood protected from the fungus physically (e.g., the cell cap, cleaning by nurse bees), or chemically (e.g., fungistatic compounds), or by high brood temperatures, or other factors?
- 2) For how long after application is the fungus effective? We usually observed spikes in mite fall 7-10 d after each application, implying that an application only infected mites on a large scale for 1-2 d.
- 3) Which part of the varroa population is being attacked by the fungus? If varroa in capped brood cells are protected (like brood), and phoretic mites are not being significantly affected, is the fungus attacking mainly emerged, non-phoretic mites? If so, what is the impact on varroa population dynamics?
- 4) How can we improve formulation or application method and still have an economically-viable treatment? We formulated most applications with inexpensive plant wax powder to help distribute conidia within the hive, and we used small amounts of conidia (0.5-1 g per application), so cost per application was low. But we also needed at least 3 applications to reduce varroa fall. Studies with strips used much more conidia = higher costs. Is there a way of increasing application half life without increasing danger to the bees themselves?

Varroa reproduction in honey bee colonies: Interactions between host and parasite and possibilities for a biological control.

Rosenkranz Peter *, Frey Eva, Odemer Richard, Ziegelmann Tina

Apicultural State Institute; University of Hohenheim August-von-Hartmannstrasse 13, Stuttgart, Germany

* Author for correspondence: E-mail: peter.rosenkranz@uni-hohenheim.de

The reproduction of the female Varroa mites within the sealed brood cells represents a crucial aspect for the population dynamic of the parasite and, therefore, for damages to the host colony. The reproductive cycle includes host finding by the female mite, activation of mite's oogenesis, egg laying, ontogenetic development of the Varroa offspring, brother-sister mating within the brood cell, and the preparation of the phoretic female mites for the next reproductive cycle.

Varroa reproduction is closely synchronized with the development of the host larvae. Several host factors have been proved to control the course of Varroa reproduction. Certain cuticular substances of the bee brood are involved in the host finding, the activation of mite oogenesis and even in the sequence of sexes of the Varroa offspring. We have also analyzed the mating behaviour of the mites within the sealed brood cell which is triggered by a female sex pheromone.

However, there are still crucial gaps in our knowledge on mite reproduction:

- We do not have a standardized method for the quantification of reproductive parameters (i.e. fertility, fecundity, reproductive rate). This is a prerequisite for the comparison of Varroa reproduction between different host and/or parasite populations (and the selection of honey bee colonies with low Varroa reproduction).
- A certain percentage of female mites remain infertile after invading a brood cell. The reasons for this temporary infertility are completely unknown.
- Obviously, successful mating is a prerequisite for the survival of the daughter mites. The reasons for this phenomenon are yet unknown.
- There exist also inhibitors of mite reproduction. However, so far only one active substance has been tested in detail.

Currently, there is no biological treatment available for the control of Varroosis. However, the above listed open questions on mite reproduction offer possibilities for such a biological control. The prerequisites for successful attempts in this research field will be discussed. Presently, the disruption of the mating of the Varroa daughter mites seems to be the most promising approach.

Diagnosing Varroasis

Sammataro Diana

USDA-ARS Carl Hayden Honey Bee Research Cntr Tucson, Arizona USA

Author for correspondence: E-mail: Diana.sammataro@ars.usda.gov

Phone: 520 670 6380 ex 121

In order to treat Varroa destructor in honey bee colonies, the number of mites present in a colony must be diagnosed. There are several ways to determine mite loads: 1. Collecting mites in a sugar/ether roll, 2. Examining the brood or 3. Using a sticky board. The accuracy of these methods will be outlined and the predictive measures and treatment times from these methods will be discussed.

Co-evolution between *Varroa* and *Apis cerana* populations: does it exist, and can we make use of it?

*Smith Deborah¹, Rueppel Olav², Warrit Natapot³

¹University of Kansas Dept. Ecology & Evolutionary Biology/Entomology, Haworth Hall, 1200 Sunnyside Ave, Lawrence, KS USA

²University of North Carolina, Greensboro

³Chulalongkorn University

* Author for correspondence: E-mail: debsmith@ku.edu 1-785-727-0317 (cell); 1-785-864-4340 (office)

Several lines of evidence suggest that local populations of *Varroa* are adapted to reproduce on their local populations of *A. cerana*, and that mite reproduction is suppressed if they invade the "wrong" host population. We are investigating the natural host-parasite system to uncover genes in *A. cerana* that may suppress reproduction of all but the adapted or co-evolved population of *Varroa*.

First, surveys of mitochondrial DNA (mtDNA) variation in *Varroa* (Anderson & Trueman 2000) revealed that the mites formerly called *V. jacobsoni* constituted several cryptic species. The geographic distribution of these *Varroa* species corresponds broadly to the geographic distribution of mitochondrial lineages within the host, *Apis cerana* (*Smith* & *Hagen 1996*): *V. destructor* is found on the Mainland Asian mtDNA lineage of *A. cerana*, *V. jacobsoni* on Sundaland *A. cerana*, and the asyet unnamed Philippine *Varroa* on the Philippine lineage of *A. cerana*.

Second, each species of *Varroa* comprises several allopatric populations, each characterized by a different mtDNA haplotype. Only two haplotypes, "Korea" and "Japan" have colonized *A. mellifera*.

Third, when *Varroa* were intentionally or unintentionally introduced to novel *A. cerana* hosts, the mites did not successfully reproduce.

We focus our work in Thailand, where allopatric populations of the Mainland and Sundaland lineages of *A. cerana* occur naturally, and where *V. destructor* (Vietnam haplotype), *V. jacobsoni* (NorthThai and Malay haplotypes) and *V. underwoodi* are present in nests of their *A. cerana* hosts (Smith & Hagen 1996, Warrit et al 2006). In addition, introduced *A. mellifera* and their *V. destructor* (Korea and Japan haplotype) are also present. Our first goal is to test, by reciprocal transfer of mites, the hypothesis that populations of *Varroa* are adapted to reproduce on their co-evolved host populations, and that they are unable to reproduce, or reproduce poorly, on populations to which they are not adapted. If the initial hypothesis is correct, our second goal is to map genes in *A. cerana* that are responsible for suppression of reproduction by non-adapted mites.