… TOWARDS RESILIENT HONEY BEES…

RESEARCH ROADMAP 2016-2026

December 2015

Photo N. Carreck 24 Nov 2015

Coby van Dooremalen, Bees@wur, Wageningen UR, The Netherlands

Annet Zweep, Department of Agro and Nature Knowledge the Hague, Ministry of Economic Affairs, The Netherlands

A co-creation by Bees@wur and the Dutch government, and the (inter)national researchers participating in the workshop Resilient Honey bees 23-24 November 2015, The Netherlands.

INTRODUCTION

THE NEED FOR RESILIENT HONEY BEES

Pollination by honey bees (*Apis mellifera*) is essential to crop and seed producers. Most of the high-quality crops that will supply our growing population with essential minerals and vitamins depend—at least in part—on bees [Aizen et al. 2009; Gallai et al. 2009; Eilers et al 2011]; in Europe alone, 84% of all crops are animal-pollinated, and 4000 varieties exist thanks to bee pollination [Williams 1996]. The value of pollination of food crops approximates 153 billion euros annually [Gallai et al. 2009]. Healthy bees are required to increase crop and seed production to feed the increasing world population and maintain the market position of these producers.

For optimal honey production and reproduction of colonies, healthy bees are essential. Costs and efforts of treating honey bee pests and diseases have increased for beekeepers, particularly since the introduction of the ectoparasitic mite *Varroa destructor*. This now mite, an invasive species from Asia, is the primary biological cause of colony mortality worldwide (Neumann and Carreck 2010, Potts et al. 2010b). This is mainly because it is a very efficient vector of several honeybee viruses, generating a disease epidemic within the colony, which dwindles until it dies (Neumann et al. 2012). The treatment costs against this mite themselves can even exceed the income generated by the colony [Potts et al. 2010a] and parasite thresholds above which damage or losses occurs are very low in common beekeeping practices [Genersch et al. 2010; Rosenkranz et al. 2010; Van Dooremalen et al. in prep]. Sustainable and easily applicable measures to control pests and diseases are currently lacking, and new approaches are urgently needed, given that stressors will likely remain in the future [Dietemann et al. 2012].

For decades, honey bee colonies experience high losses during winter, especially in the Northern hemisphere, and these losses are still continuing in some countries [VanEngelsdorp and Meixner 2010; Neumann and Carreck 2010; Lee et al. 2015]. Much effort has been put in research to understand why and how these losses occur. To date, colony losses are likely due to several simultaneously occurring stressors [Genersch et al 2010; Alaux et al. 2011; Van Dooremalen et al. 2013], and often involve pests and diseases [Neumann and Carreck 2010; Potts et al. 2010b], and very often the pest *V. destructor*. While the primary aim to solve the colony losses was to avoid crisis on the short term and mitigate losses using all means possible, a new trend is emerging with more focus on long term investment in resilience of honey bee colonies.

Resilient honey bees are a necessity to solve the honey bee problems of today and to work towards sustainable beekeeping in the future. Resilient honey bee colonies are (locally) adapted to be less susceptible and more robust under high infection pressures either by resistance against or tolerance for pests or diseases. The availability of resilient colonies will reduce costs associated with treating pests and diseases, help to reduce losses, and thereby make beekeeping more (cost) attractive. Boosting the beekeeping sector is required, as currently the global stock of domesticated honey bees is growing slower than agricultural demand for pollination [Aizen and Harder 2009].

WORKSHOP IN THE NETHERLANDS

In 2013, an Action Program to improve on Dutch Bee Health was prepared by the Dutch beekeeping and agricultural sectors and related organizations (100 representatives, including Bee@wur) and offered to the State Secretary for Economic Affairs. One of the core theme's from the program was 'pests and diseases in bees', where *Varroa destructor* and related viruses, *Nosema* spp., Chalkbrood, as well as American and European Foulbrood were prioritized to work on together with newly emerging pests or diseases. Within the theme Pests and diseases in bees, three approaches were called for: 1) select for resistance of bees to *V. destructor* and other pests and diseases (make the bees more resilient), 2) develop new sustainable control methods, and 3) clear, fast and effective dissemination of new knowledge to the sector.

In line with the Action Program, Bees@wur started in 2014 with the project Resilient Honey bees[1], supported by the Dutch Ministry of Economic Affairs. Since, a report has been written (in Dutch), giving the state of the art on pest and disease resistance and listing the (inter)national initiatives within this field of expertise [Blacquière 2015]. A selection of key players of these initiatives was invited to participate in a workshop to set the research agenda on Resilient Honey bees on 23-24 November 2015 in The Netherlands (Appendix 1).

The aim of the workshop was to bring together the state of the art of research on resilient honey bees, to exchange ideas, define knowledge gaps, and to align (inter)national research programs where possible. The outcome of the workshop in combination with information from the report was the basis for this roadmap.

[1] Currently, this (workshop)project 'Resilient Honey bees' runs parallel to the Project on Varroa resistance/tolerance in Dutch Honey bees, led by Tjeerd Blacquiere. The 'Varroa project' started in 2007 and is financed by the EU and the Dutch Ministry of Economic Affairs. It aims to develop a concept to obtain Varroa resistant/tolerant bees by means of natural selection [Blacquiere et al. unpublished data].

STATE OF THE ART

Using the knowledge of the existing research, a conceptual framework for the road towards resilient honey bees can be designed (Figure 1).

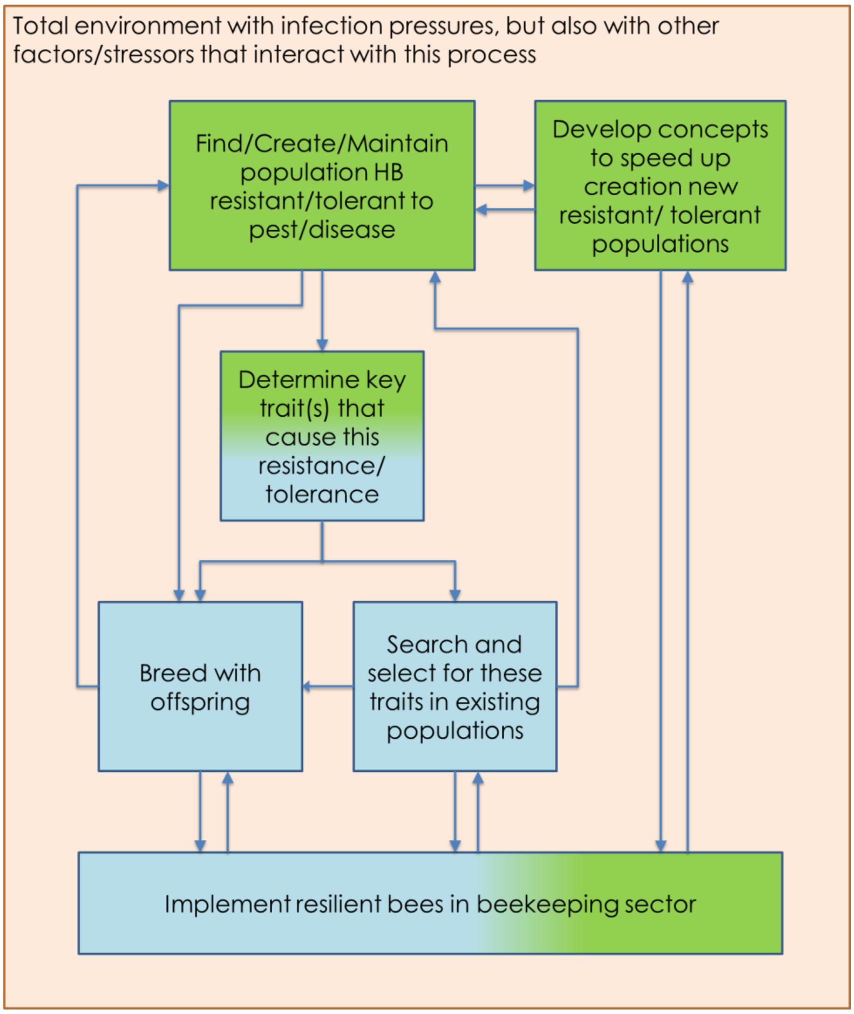

Figure 1. Conceptual framework for the road towards resilient honey bees (HB = honeybees).

This framework was used during the workshop. Research and initiatives within the green blocks in the figure try to create resistant populations of honey bee colonies using (semi) natural selection or try to unravel the underlying mechanisms that cause this resistance. Research and initiatives within in the blue blocks breed honey bee colonies using selection for specific traits that were previously identified to (partly) cause resistance in existing resistant populations. Research within in the outer orange block investigates the effects of environmental factors that may affect or interact with this process, including the way beekeepers manage their bees. For the position of the participants within the framework, see Appendix 1.

Using our knowledge on breeding and natural selection for (local) adaptation to pests and diseases, we can work towards honey bee colonies that are less susceptible and/or more robust. That is necessary because e.g. the parasite *V. destructor* has high potential to become resistant against the most common acaricide treatments (abstract 19, Appendix 2) and has become resistant to some of them already.

By natural selection, adaptation in honey bee colonies exposed to (high) pathogen pressure will result in populations that will survive without treatment [Locke 2015]. At this moment there are for example several of such populations that show resistance against the parasite *V. destructor* (abstracts 1, 5, 6, 15; reviewed by Locke 2015). From there, we can either develop concepts that enable us to repeat creating new resistant populations at other locations (abstract 6, 7) or work from these resistant populations (abstract 13). At the same time, the underlying mechanisms and traits that cause this resistance have partly been unravelled (abstracts 2, 3, 4, 8, 9) to better understand the evolution of the host-parasite relation.

By introducing (mated) queens from the resistant population into other populations the resistance of these populations may increase, with or without knowing exactly which traits are responsible. Knowing the traits (partly) responsible, makes it possible to search and select for these traits in other existing populations that are not resistant or may not survive without treatment yet. Furthermore, understanding the underlying mechanisms allows for better management of resistant populations. Several breeding programs to improve resistance against for example *V. destructor* have been set up (abstract 10, 11, 13, 14) and supporting tools are being developed (abstracts 8, 12).

The environment, in which these populations exist, increases the complexity of the process to obtain resilient honey bees (abstract 16). Selection depends on the intensity of pest or disease stress to which the colonies are exposed (selection pressure) and on the local conditions (abstract 15, 17). But also some environmental stressors have been identified to potentially interact with this process, i.e. neonicotinoids affecting individual immunity of bees (abstract 18), which may decrease bee health.

The environment also includes the beekeepers that manage the bee colonies. The geographical distribution and density of beekeepers within the different areas that all have their own breeding strategies will further increase the complexity. Beekeepers that treat their colonies against mites reduce the selection pressure by removing the pest, while beekeepers that stop treating increase the chances of re-infestation of colonies just treated against mites.

The final part of the conceptual framework is the implementation of resilient honey bees in the beekeeping sector. In The Netherlands, some progress has been made and initiatives have started (bottom up) from within the sector itself either based on creating a new resistant population by selecting on survival and growth of the colony (abstract 7), by breeding with material from existing resistant populations (abstract 11, 13), or by selecting for a particular trait (abstract 13) in existing populations (abstract 11).

AGENDA TOWARDS RESILIENT HONEY BEES

There is great concern about the health and resilience of our presently kept honey bees. Although resilient honey bees should ideally be resilient to the widest variety of pests and diseases possible, these bee colonies will never be able to cover the whole spectrum at once. There was major consensus on *V. destructor* and associated viruses being the most important pests to tackle at this moment. Consensus exists that the resilience of bees needs to be increased to avoid the use of drugs/chemicals/acaricides.

From the state of the art and the discussions during the workshop (see appendix 3 for summary of workshop discussions) we distinguish between two approaches that may lead to resilient honey bees: a) concepts based on natural selection and b) breeding programs selecting for specific traits. These two approaches are not mutually exclusive; provided that at least the mating stations of both approaches are kept separate (table A3.5 in Appendix 3). To a certain extent the breeding approach utilizes natural selection forces and also can benefit from the natural selected populations of resilient colonies to help determine the key traits causing resilience needed for breeding programs. On the other hand, the natural selection approach can use classical selection to artificially select for e.g. production traits or can benefit from classical breeding to re-introduction of e.g. production traits that may be lost during the bottle neck.

From the environmental viewpoint it is essential that both approaches maintain a holistic and integrated approach taking into account the beekeepers, but also potential effects of interactive stressors. It was noted that what is good for Varroa resistance may facilitate other diseases that need other conditions/environments. Many diseases are however vectored by *V. destructor* and are expected to reduce with increased *V. destructor* resistance. Moreover, several (behavioural) traits acting against *V. destructor* are effective against other diseases too. At the same time, efforts should be made to reduce as much as possible bee exposure to unnecessary stressors (i.e. agricultural stressors or beekeeping stressors that originate from mismanagement or misuse).

Maintaining honey bee resilience in the future depends on monitoring and anticipation towards new threats invading the honey bee environment. We should keep our eyes open for such threats like new invasive species and global climate change.

GENERAL AIMS FOR BOTH APPROACHES

For this agenda, we will first address some general aims and objectives that are an umbrella for all approaches (both current ones, but also future ones) and that need to be considered in order to achieve the central goal of resilient honey bees.

<u>Short to mid-term aims/objectives</u>

- Find a balance between fundamental and applied research. In this light, there are some fundamental questions that still need to be clarified in the future:

- How does the pathogen (pest or disease) affect the colony as a whole and what are the trade-offs involved?
- How can a colony become resistant?
- How do resistant colonies perform under the day-to-day variation in their local environment and under local apiary management?

There are also some applied questions that still need to be clarified in the future in order to progress from the promising current pioneer and piloting initiatives to generally accepted protocols that are incorporated into common practice. Several initiatives are already working on pest or disease resistant honey bee colonies, all using their own approaches, methods and protocols, but overall we need to answer:

- How can we maintain the population's resistance within a genetically heterogeneous landscape? If difference approaches lead to different strategies within populations, and these populations occur side by side, how to preserve these population's own characteristics (resistance traits)?
- How to conserve the genetic basis at the geographical location where it is supposed to be? This question has a strong link to the short discussion we had on transgenic honey bees (see Box in Appendix 3).

o Commit beekeepers, as they are essential stakeholders to incorporate resilient honey bees into common practice, independent of their preferred approach. To gain this commitment it is important to maximally share the knowledge about current initiatives (maximize extension). Knowledge should be disseminated, but beekeepers can also actively be involved in pioneering and piloting work. Beekeepers are already involved to some extent in e.g. local or national breeding programs (abstracts 8, 9, and 10) or have set up their own initiatives (abstracts 7, 11, and 13, Appendix 2). More objectives to involve beekeepers that were discussed during the workshop will be given in the two separate paragraphs on the 'natural selection' and 'breeding programs' approach below.

Continuous aims and objectives (short, mid, and long term)

o Resilient bees should ultimately not only be resistant to a single pest, but be robust over a broader spectrum. Therefore it is important to take into consideration in every approach that stressors inevitably act in combination (*V. destructor* and X). Bees (including managed and wild bees, social and solitary bees) are subject to numerous pressures in the modern world: exposure to cocktails of agrochemicals, various pathogens, lack of abundance and diversity of feed, flowers and climate, but are also subject to socio-economic factors and human behaviour, including beekeeper compliance and stakeholder interests (Table A3.2 in Appendix 3).

o In order to function optimally, bees should fit into their local ecosystem. In contrast to other livestock, bees are intimately interwoven into ecosystems. There are no two locations which share a combination of environmental factors. Each site is unique, not only varying in the combination of stressor exposure, socio-economic factors and human behaviour, but also in the general abiotic factors (e.g. climatic conditions, landscape type, water availability) and biotic factors (e.g. genetic bee pool, land use, food availability, animal and plant communities). For each approach, programs should take account of genotype-by-environment interactions and protect these in time and space (Table A3.2 in Appendix 3).

NATURAL SELECTION

We determined the short-term (until 2021), mid-term (until 2026) and long-term (beyond 2026) objectives and aims for this approach. These aims/objectives were derived from the challenges determined for the natural selection approach during the workshop. For more detail, please see Tables A3.2 and A3.3 (Appendix 3).

Summarized:
For the natural selection approach, concepts to start up new potential resilient populations need to be developed, refined and tested for implementation into beekeeping practice. Infrastructures need to be put in place. Involvement of groups of adaptive beekeepers is essential in order to create 'isolated' or focus areas for reproduction and to let beekeeping practice co-evolve with the 'new colony phenotype'.

<u>Short term objectives and aims</u>

- Better understand the fundamental host-parasite relation (incl. variation in pest infestations), current existing resistant/tolerance mechanisms and related trade-offs (continuous aim for mid and long term)
- Test the general performance (incl. trade-offs) of the resistant colonies resulting from this approach to predict the feasibility of implementation into the sector
- Develop (new) concept(s) to repeat selection
- Involve beekeepers in the development of the concept(s) and pilot(s) to create commitment and support
- Explore potential of these concepts to counteract other invasive species (e.g. small hive beetle)

<u>Mid-term objectives and aims</u>

- Start to implement concepts in beekeeping sector
- Stop using acaricides when and where possible
- Test/monitor the general performance (incl. trade-offs) of the resistant colonies in the sector to evaluate the implementation of resilient bees
- Proactively develop new strategies to deal with invasive species

<u>Long term objectives and aims</u>

- Fine-tune protocols for regional, apiary and colony management to maximize sustainability of the resilient population
- Be able to predict the host-parasite relationship (model)
- Have an increasing number of new beekeeper initiatives that incorporate the concept of natural selection for *V. destructor* resistance into their common practice and that have their own locally resistant honey bees
- Decrease acaricide use to the minimum

- Match objectives of beekeepers with resilient bee needs for a sustainable future of resilient bees

Involvement of committed local groups of beekeepers herein is essential as most likely beekeeping practices need to adapt to new honey bee colony phenotypes and adapt to a decreased use of acaricides. Objectives to involve beekeepers in the natural selection approach:

- Facilitate initiatives such as VBBN Laren (abstract 7 Appendix 2)
- Use experience Bees@wur with the *V. destructor* brochure to develop a sort of cookbook for the natural selection concept
- Internet platform to motivate and inspire beekeepers to take initiatives to stop treatment and start selecting for resilient honey bees.
- Involve beekeepers in science projects (e.g. CSI pollen within the COLOSS network; www.coloss.org). Internet platform (per country) to get knowledge to beekeepers about the need and methods of locally organized selection for natural *V. destructor* resistance.

BREEDING PROGRAMS

We determined the short-term (until 2021), mid-term (until 2026) and long-term (beyond 2026) objectives and aims for this approach. These objectives and aims were derived from the challenges determined for the breeding program approach during the workshop. For more detail, please see Tables A3.2 and A3.4 (Appendix 3).

Summarized:

For the breeding approach, existing breeding structures need to be refined and enforced and additional new breeding structures need to be put in place. Tailored breeding and selection tools need to be developed and be made available to beekeepers. There is a continuous need for new technologies, but also for new drugs to bridge the gap between current practices and future resilient bees. Involvement of beekeepers is essential in order to map high potential breeding material in those beekeepers' stocks and to determine the 'wish-list' of the beekeepers' required bee traits.

Short term objectives and aims

- Exchange experiences in breeding to design optimal local solutions
- Propose breeding tools like databases, cryopreservation, practical, standardized, selection tools and protocols to maximize breeding output
- Make standard protocols for breeding and training of breeders

Continuous objectives and aims (but start at short term)

- Develop new treatment or treatment application strategies (treatment needed for many years to come), including strategies where colonies with low infestation levels are not treated, to overcome the time until reaching complete pest or disease resistance
- Support local breeding structures to allow implementation of local and global developed solutions.

- Support mating control (mating stations, instrumental insemination) to allow development of alternative and local solutions.
- Support initiatives where measurement of *V. destructor* infestation is linked to propagation, leading to "genetic death" of *V. destructor*-sensitive genotypes i.e. all colonies that show an increase in mite population need to be removed from the population without exception (which differs from the natural selection approach where colonies may remain if they survive and grow in bee-numbers in spring).
- Encourage cooperation between beekeepers, which is also important to multiplication/distribution of solutions.
- Facilitate close cooperation with research and beekeepers to ensure balanced traits in colonies

Mid-term objectives and aims
- Optimize beekeeping systems for local use in combination with selective breeding by finding easy traits and find markers for selection
- Develop different (standardized) tools that best meet local and/or level of breeding experience
- Continue developing molecular methods to facilitate breeding
- Test the general performance (incl. trade-offs) of the resistant colonies resulting from this approach to predict the feasibility of (further) implementation into the sector
- Integrated pest management (threshold based, including resistance management) for the intermediate term, to work towards *V. destructor* control without treatment

Long term objectives and aims
- Increase our understanding of relevant viral and bacterial diseases at low *V. destructor* infestation. Selection for stronger antiviral response may support overall resilience

Involvement of committed beekeepers herein is essential to map high potential breeding material and to determine the specific traits to select for. Objectives to involve beekeepers in the breeding program approach:
- Invite experienced beekeepers to participate in breeding programs such as AGT (abstract 10), Arista Bees (abstract 11), Duurzame bij (abstract 13), or Beebreed (for abstracts see Appendix 2)
- Offer services and get something back (e.g. service measurement of resistance of colonies and collect data on traits)
- Use monitoring programs to make an inventory of the wishes of the beekeepers. E.g. In Germany and other countries standard monitoring was extended by questions like 'What are your needs with respect to education and/or beekeeping?'
- Make standard protocols for breeding and training of breeders. The book '*V. destructor*, still a problem in the 21st century?' already explains the ins and outs of breeding for beekeepers. Standardizing of methods was done already in the

COLOSS BEEBOOK (i.e. breeding, including measurement of traits and rearing) and the beebook platform, but the beebook was written for scientists. Within the EU SmartBees project scientists are now working on a master version of a 'beekeeper/breeder beebook' (translating the Beebook breeding chapters into a beekeeper-friendly booklet), which is expected in 2017

- Organize workshops with breeders to implement and locally adapt breeding protocols. This could be an example for a breeding structure
- Make an internet platform (per country) to get knowledge to beekeepers about standardisation of breeding strategies.

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APPENDIX 1. PROGRAM AND LIST OF PARTICIPANTS

Version 2015-11-19

Day 1

09:00 Welcome – Coby van Dooremalen

The need for resilient bees from the sector –Jan Dommerholt, Dutch beekeepers association

Context and aim workshop – Annet Zweep

Program and household remarks

10:00 Presentations **Natural selection and Biology** – 7 x 10min talk (coffee break in between) followed by one plenary discussion of 30min.

12:15 Lunch + walk (weather permitting)

13:00 Presentations **Breeding Programs** – 6 x 10min talk followed by one plenary discussion of 30min.

15:00 Break coffee/thee

15:15 Presentations **Environment** – 5 x 10min talks followed by one plenary discussion of 30min.

17:00 Buffer time / free time

18:00 Dinner

Networking and socialising (bar is open)

Day 2

09:00 Welcome and program

09:15 Parallel discussions on Biology, Breeding and Environment: knowledge gaps, ambitions, and priorities within the topic

10:30 Break coffee/thee and chairmen will have time to prepare

11:00 Plenary summary of the parallel sessions by the chairmen (max 10min talks) followed by one plenary discussion (60min): the context, our needs to realise ambitions on short and long term

12:30 Lunch + walk (weather permitting)

13:30 Strategic agenda for resilient bees: knowledge gaps, possibility of alignment and collaboration

15:15 Wrapping up (15min)

16:00 Early dinner (optional)

The workshop participants. The list shows the name of the participants and co-authors, the country, and the title of the talk as presented during the workshop. The colours in the first column coincide with the colours in the conceptual frame work for the road towards resilient honey bees in figure 1. The numbers coincide with the abstract numbers in Appendix 2.

Nr.	Name participant and co-authors	Country participant	Title workshop talk
1	Fanny Mondet, Alison Mercer, Yves Le Conte	France	Varroa surviving honey bees in France and mechanisms of VSH behaviour
2	Bart Pannebakker	Netherlands	Insights into the genetic basis of Varroa tolerance in European honey bee populations
3	Jarkko Routtu, Robin Moritz	Germany	Dynamics of a novel host-parasite coevolutionary arms race
4	Peter Rosenkranz	Germany	Reproduction under time pressure: Factors affecting the reproductive success of female *Varroa destructor*
5	Joachim de Miranda, Ingemar Fries, Barbara Locke	Sweden	The Gotland 'Bond' varroa-resistant honey bee population
6	Tjeerd Blacquière	Netherlands	Naturally selected honey bee colonies can cope with varroa
7	Pam van Stratum	Netherlands	Vital bees have the future
8	Jakob Wegener	Germany	Research towards resilient bees at the LIB
9	Lilia de Guzman, Thomas Rinderer	United States of America	Breeding varroa-resistant bees: The search for a new selection tool
10	Marina Meixner, Ralph Büchler	Germany	Coordinated selection and breeding efforts improve the mite resistance of honey bee populations
11	BartJan Fernhout	Netherlands	Breeding Varroa resistant honey bees in the footsteps of John Harbo
12	Pim Brascamp	Netherlands	Optimisation of breeding programmes in honey bees
13	Marleen Boerjan, Henk Kok, Gerrit Plas, Egbert Touw, Job van Praagh	Netherlands	The foundation De Duurzame Bij fights Varroa mite sustainable
14	Dirk de Graaf	Belgium	Efforts to refocus Flemish breeding initiatives *(no talk during workshop)*
15	Peter Neumann, Bjørn Dahle, Vincent Dietemann, Dirk de Graaf, Joachim de Miranda, Lina de Smet, Ingemar Fries, Yves Le Conte, Barbara Locke Grandér, Peter Rosenkranz, Jarkko Routtu, Robin Moritz	Switzerland	The lord of the rings: European honey bees surviving varroa by means of natural selection.
16	Johannes Wirz	Switzerland	Towards a Resilient Bee
17	Norman Carreck	United Kingdom	What future for local bees in Britain?
18	Annely Brandt, Marina Meixner, Ralph Büchler	Germany	How to measure resilience? The immune system as focal point of honey bee health
19	Robin Moritz	Germany	Selection of acaricide resistance in Varroa and the need for resilient honey bees.

APPENDIX 2. ABSTRACTS

1. Varroa surviving honey bees in France and mechanisms of VSH behaviour

Fanny Mondet1, Alison Mercer2, Yves Le Conte1
1 INRA Abeilles et Environnement, Avignon, France
2 Department of Zoology, University of Otago, Dunedin, New Zealand

For several years, the INRA has been maintaining two populations of local honey bees that survive varroa mite infestations in the absence of any treatment. This natural selection program started in the mid 90's, with the observation that some colonies survived in abandoned apiaries. These surviving populations have low development rates of varroa during the season. Since early 2000, our research is aimed at understanding the origin of the resistance of these populations, with focus on unravelling the basis of chemical communication between honey bees and varroa mites.

More recently, priority was given to the study of chemical communication around VSH behaviour. We are currently conducting research to address the following: how do bees detect the presence of varroa in the colony, and more specifically in the brood? Our first results confirm the primary role of olfaction and of the peripheral nervous system in the ability of adult bees to develop VSH behaviour.

2. Insights into the genetic basis of Varroa tolerance in European honey bee populations

Bart Pannebakker
Laboratory of Genetics, Wageningen University, The Netherlands
e-mail: bart.pannebakker@wur.nl

The Laboratory of Genetics investigates causes and consequences of natural genetic variation within species. Our aim is to understand the interrelationship between genetics, heredity, and evolution to explain patterns of biological variation. Our honey bee work focuses on the genetics of the relationship between honey bees and the Varroa mite, the major pest of honey bees. We aim to understand the genetic basis of Varroa tolerance in honey bees. This will not only provide a better insight into the mechanisms of Varroa tolerance, it will also help us explore potential unwanted side-effects, and ultimately help to develop markers for marker-assisted selection. Our focal point is the genetic basis of Varroa sensitive hygiene (VSH) behaviour in European honey bee populations, in which honey bees remove mite infested larvae and pupae from sealed brood cells.

I will present our work on the genetic basis of Varroa tolerance in European controlled and natural honey bee populations. Using a molecular genetic approach, we identified several candidate regions for VSH behaviour that we are currently testing for further polymorphism in controlled and natural populations. Our future work will concern detailed quantitative trait locus analyses of VSH in European populations, in order to further pinpoint the causative variants underlying this important trait.

3. Dynamics of a novel host-parasite coevolutionary arms race

Routtu, J. & Moritz, R.F.A.

The aim of our project is to find genetic basis of honey bee drones resistance to *Varroa destructor*. Drone resistance to Varroa has been the emerging causal trait in natural selection based experiments to produce Varroa resistant colonies. As the Varroa mite has strong preference to breed in drone brood it is not surpricing that resistance first emerges in drones. The project will be done by using QTL crosses in combination with high density SNP linkage maps and haploid genomes of the drones. This approach will be complemented with RNA-seq and proteomics datasets. The results will be important in understanding how novel host-parasite interactions coevolve and will provide practical knowledge for apiculture with Varroa resistant honey bees.

4. Reproduction under time pressure: Factors affecting the reproductive success of female *Varroa destructor*

Peter Rosenkranz
University of Hohenheim, Apicultural State Institute, August-von-Hartmannstrasse 13, D-70599 Stuttgart, Germany
peter.rosenkranz@uni-hohenheim.de

The reproductive success of female *Varroa destructor* is limited by the duration of the post capping period of the brood. During the time period of approximately 12 days the foundress mite has to activate oogenesis of at least two eggs, perform embryogenesis and fertilize the female eggs. The male mite has to produce up to 150 mature sperms in order to mate with one or more female daughter mites. We present data on the time course of the development of male and female eggs, the capacitation of spermatozoa, the mating behavior, and the phoretic phase. Based on these data we estimate the possible reproductive rates of female mites and the consequences for the Varroa population dynamic. We discuss the possibilities and requirements to use reproductive parameters of the mite for the selection of resilient honey bees.

5. The Gotland 'Bond' varroa-resistant honey bee population

JR de Miranda, I Fries, B Locke

Between 1997 and 1999, an experimental honey bee population of 150 varroa-free colonies with diverse genetic backgrounds was established on an isolated peninsula on the southern tip of Gotland, in the Baltic sea, and infested with mites. The original purpose was to determine the effect of swarming on varroa population development. However, after the expected population crash in the 3rd year of the experiment, there remained a small, resilient bee population that has persisted to this day without any varroa management. This surviving population has been the subject of numerous biological, genetic, genomic and pathological studies over the years, in order to identify the nature of this adaptation. The adaptation lies with the bees, rather than with the mites, and includes changes to the bee population growth, a reduced reproductive success of the mite on bee pupae, and changes to pathogen profile. Current research focuses on the molecular determinants of the, evolutionary-adaptive process, the reduced mite reproduction and metagenomics changes in this population.

6. Naturally selected honey bee colonies can cope with varroa

Tjeerd Blacquière
Bees@wur, Wageningen UR, PO box 16, 6700 AA Wageningen, The Netherlands
e-mail: tjeerd.blacquiere@wur.nl

Because varroa is the most serious threat to beekeeping, but colonies left alone survive in nature, we set up a group of colonies in which varroa was not controlled since 2008 (WD). Part of the group was kept as a control which was managed similarly apart from two varroa control treatments per year (C). In addition we followed a population on the island Tiengemeten with partial ancestry from Gotland (2005) (TG).

- During early summer well performing colonies which have produced ample drones are split into ~4 nukes with a young offspring emergency queen (Queen had been removed 2 weeks before);
- the nuke queens are mated in a remote area;
- fast grown colonies reaching an acceptable size are wintered;
- colonies surviving after winter go for the next round: reproduce or be removed.

After initial losses the population of colonies is stable. Varroa infestation levels vary between 5 and 15% (phoretic, when no brood is present). The varroa population is growing slower than in the control group.

Reproduction of mites: share non-reproducing mites increased (especially TG), N viable female offspring was reduced. More often the male was absent.

Grooming activity in general (dust) and against mites in laboratory assays as well as the loss of phoretic mites at the colony level was reduced in both selections, but no effect on number of injured mites was found.

VSH was present in C, reduced in TG selection, but strongly increased in WD. Now focussing on the effect of VSH on DWV/VDV-1.

It looks as if the wisdom of the hive should be allowed to extend to choice of genes instead of leaving that to the queen breeder.

7. Vital bees have the future

Pam van Stratum
Inbuzz Extra, IJsbaanweg 8, 1251, VV Laren

With our local beekeepers association, working group 'Vital bees', we maintain a bee population that is not treated against Varroa destructor since June 2009. The goal of our experiment is to obtain a population of honey bees that tolerate Varroa infestation, using a beekeeping method suitable for each beekeeper or group of beekeepers. The essence of the method is guided natural selection, in which mites do the selection job. In order to maintain the existing genetic variation in bees, we consider that it is important that local available bees are used.

Our beekeeping method is a colony contest: every year at the end of spring, the largest colonies are split in new colonies with young queens. We make sure that our young queens are sexually mature at the same time as our drones, to reduce influence of 'foreign' drones. These new colonies may grow and the following year we repeat the procedure with the new largest colonies.

Twice a year Varroa density is measured to monitor how Varroa tolerance is developing. Note that these data are not used for selection. Only colony size matters.

As a result of the successful selection, we now maintain, parallel to the selection contest population, a year round apiary in order to ensure honey production throughout the season.

8. Research towards resilient bees at the LIB

Jakob Wegener, Länderinstitut für Biennekunde Hohen Neuendorf e.V., D-16540 Hohen Neuendorf; wegenerj@hu-berlin.de

My talk will give a brief overview of past and present activities of the breeding&genetics-group at the Hohen Neuendorf Bee Institute that aim at the selection/protection of resilient bees, and identify areas for future research:

Colony-level pathology, specifically the mechanisms by which Varroa destructor + viruses damage colonies. First results from a recent study illustrate that insect-level effects can rarely be extrapolated to the colony, so in order to identify possible tolerance mechanisms, a better understanding of the sensitive points of colony physiology is needed.

Application of quantitative genetics to the problem of selecting resilient bees. Here, our activities within the EU-project SmartBees include the creation of subspecies-specific databases and algorithms of breeding value estimations. In parallel, another project deals with the integration of data from genomic analyses.

Genetic markers for resistance traits. Here, we are working with partners within SmartBees to create a set of SNPs associated with hygienic behavior, using bees of different subspecies.

Cryopreservation of honey bee genetic resources (also for support of in situ-breeding programs). We have recently presented an improved protocol for semen storage, and plan to expand the technology to diploid genomes.

We are looking for partners for several of these areas, and welcome suggestions for cooperation.

9. Breeding varroa-resistant bees: The search for a new selection tool

Lilia I. de Guzman and Thomas E. Rinderer

Honey bees that are resistant to varroa mites have been selected based on either low mite population growth or Varroa Sensitive Hygienic (VSH) trait. Development of new selection tools would enhance bee breeding programs. In an attempt to find new selection tools for breeding varroa-resistant bees, we decided to evaluate the relationship between hygienic brood removal and mite removal using Russian honey bees (RHB) and Italian honey bees (IHB) as comparison. Based on a series of experiments we found that: a) RHB colonies were hygienic towards varroa-infested brood; b) brood removal greatly influenced mite removal in both RHB and IHB colonies; c) highly hygienic RHB colonies expressed high levels of grooming; d) older mites/trapped (O/T) was negatively correlated with colony mites; e) O/T was higher in RHB than IHB and was positively correlated with hygienic brood removal; and f) mite injuries were not associated with colony infestation parameters and thus, mite damage may not be the best measurement of grooming. Hence, O/T can be used to evaluate colonies for mite resistance through grooming. The value of O/T as an indicator of reduced mite population in the colony and as a tool for the selective breeding of honey bees that resist varroa through grooming is currently being evaluated.

10. Coordinated selection and breeding efforts improve the mite resistance of honey bee populations

Marina Meixner, Ralph Büchler
Landesbetrieb Landwirtschaft Hessen, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany
Email: marina.meixner@llh.hessen.de

Coordinated breeding efforts to improve mite tolerance in Germany have been implemented since 2004 in the "Arbeitsgemeinschaft Toleranzzucht" (AGT), an association of honey bee breeders. Breeders of this association evaluate several thousands of queens each year, according to a common protocol which includes resistance traits such as development of mite population during season and brood hygiene. Selection is intensified by subjecting promising colonies to overwintering without treatment ("vitality test") where colony development and mite infestation are continuously monitored between August and October. Colonies overwintering successfully without any treatment are preferably selected as breeders, while colonies in danger of collapsing are treated, but rarely used for further propagation. In addition, tolerance mating stations have been established, where drones are reared under high infestation pressure in colonies that remained untreated for a long time. Thus, natural differences in drone fitness are also integrated into the selection process.

Within the Smartbees project (www.smartbees.eu), this concept is extended to regional selection activities all over Europe to preserve local subspecies and ecotypes by improving their acceptance for beekeepers. In addition, selection is taken further by identifying breeder colonies with high levels of non-reproducing mites (SMR/VSH) and the establishment of breeding lines with increased expression of this trait.

11. Breeding Varroa resistant honey bees in the footsteps of John Harbo

Bartjan Fernhout, Stichting Arista Bee Research
Email: bartjan.fernhout@aristabeeresearch.org

The western honey bee is being challenged by a highly damaging mite: *Varroa destructor*. Treatment against Varroa gives variable results, is laborious and has not reduced colony losses to pre-Varroa levels. The United States Department of Agriculture in Baton Rouge (USDA) has selected bees that can detect reproducing Varroa and remove the infected pupae and mites from the brood: Varroa Sensitive Hygiene (VSH) behavior. Inspired by the US results, teams in Europe have started to investigate whether the VSH trait is also present in original European Buckfast and Carnica breeding stock.

Inspired by the US results, Arista Bee Research teams created in 2014 and 2015 three hundred sixty small colonies, a quarter with a USDA-VSH background and the others with either (pure European) Buckfast or Carnica background. Queens were artificially inseminated with only a single drone. After an extra infestation with Varroa mites, the colonies were investigated at the end of the summer for the fraction of non-reproducing mites in the brood, this being the key measurement for establishing the level of VSH.

74 colonies were identified with high levels (more than 50 % non-reproduction) of the VSH behavior. Half of these colonies are from the European Buckfast and Carnica background, so these results show that the VSH behavior, previously shown in US colonies, is also present in European bees.

12. Optimization of breeding programs in honey bees.

E.W. (Pim) Brascamp
Animal Breeding and Genomics Centre, Wageningen UR, The Netherlands.
Email: pim.brascamp@wur.nl

My research aims to provide tools to optimize selection and multiplication in breeding programs in honey bees. Traits of interest include honey yield, behavioral traits and Varroa tolerance. As a first step improved methods were developed to estimate breeding values, in which colony traits genetically are taken to be influenced by workers and queen. As a next step along heritablities and genetic correlations were estimated in a data set of about 15,000 colonies of an Austrian Association for Beekeepers, Biene Österreich. Results confirmed that the model including effects of workers and queen properly described the data and can routinely be used to estimate breeding values.

In the context of Arista Bee Research (selection for Varroa Sensitive Hygiene, VSH) research focuses on design of testing and selection schemes, including schemes for multiplication such that improved stock can be made available for beekeepers at large.

The latter (multiplication) seems often overlooked when designing breeding programs.

13. The foundation De Duurzame Bij fights Varroa mite sustainable

Marleen Boerjan, Henk Kok, Gerrit Plas, Egbert Touw, Job van Praagh

The foundation De Duurzame Bij accepts that honey bee colonies will be never free of *Varroa destructor* mites but realizes that mites and honey bees can live together. From research we know that some honey bee colonies can cope with the varroa mite because in these colonies the reproduction of mites is reduced by up till now undisclosed mechanisms. De Duurzame Bij was initiated by late beekeeper Ed Pieterse who imported in 2001, 12 Primorsky 'Russian' queens from the USA. Neeltje Jans was used as a mating station. In 2012-2014 Egbert Touw finally succeeded to overcome bureaucratic obstacles for the legal import of sperm and eggs from USA

‘Primorskybees’. Although De Duurzame Bij started with the proven varroa resistant Primorsky bee in 2001, we always had interest to search for varroa resistance in local bees that mainly have their genetic background in *Apis mellifera carnica, Apis mellifera ligustica, Apis mellifera mellifera* (black bees) or Buckfast bees. In 2015 we succeeded to raise 17 new Primorsky queens, and 30 ´black bees´ and for the buckfast beekeepers we had 18 fertilized queens. We still use Neeltje Jans as mating station for each session we place selected drone delivering colonies. These drone delivering colonies are tested according to the DDB-protocol for a low growth in varroa mite fall.

14. Efforts to refocus Flemish breeding initiatives

Dirk de Graaf
Honey bee Valley, Ghent University, Krijgslaan 281 S2, 9000 Ghent, Belgium

In the north of Belgium (the region Flanders) different breeding initiatives have been taken for more than a decade by the different beekeepers associations. Most of this work was financed by the so-called honey project, a EU-funded project for support of the beekeeping sector in the different member states. Depending on the beekeepers association involved, different breeding strategies have been followed: a minority of the breeders participated in the beebreed-program; the majority was involved in a larvae grafting program for restocking of the hives. In the latter, a limited number of breeders are provided with the possibility to travel to land and island mating yards in Belgium, the Netherlands or Germany with selected virgin queens. Based on their performances, these queens then can become breeder queens from which genetic material is distributed through larvae grafting in the following years. Consequently, for many years the massive propagation of a limited genetic stock has been done. With the growing awareness that the vitality of bee population partially depends on its genetic diversity, the Flemish beebreeders, together with researchers from the Ghent University are trying now to refocus the breeding initiatives. We have the ambition to include the selection for varroa tolerance herein.

15. The lord of the rings: European honey bees surviving varroa by means of natural selection

Peter Neumann[1,2,3], Bjørn Dahle[4], Vincent Dietemann[2,3], Dirk de Graaf[5],
Joachim R. de Miranda[6], Lina de Smet[5], Ingemar Fries[6], Yves Le Conte[7],
Barbara Locke Grandér[6], Peter Rosenkranz[8], Jarkko Routtu[9], Robin F.A. Moritz[9]
[1] Institute of Bee Health, University of Bern, Bern, Switzerland
[2] Agroscope, Swiss Bee Research Centre, Bern, Switzerland
[3] Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa
[4] Department of Animal and Aquacultural Sciences, Norwegian University of Life sciences, Ås, Norway
[5] Department of Physiology, Ghent University, Ghent, Belgium

[6] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[7] Abeilles et Environnement, French National Institute for Agricultural Research, Avignon Cedex, France
[8] Landesanstalt für Bienenkunde, Universität Hohenheim, Stuttgart, Germany
[9] Institut für Biologie/Bereich Zoologie, Martin-Luther Universität, Halle / Saale, Germany

For >20 years, breeding efforts towards developing European honey bee tolerance to *Varroa destructor* have not achieved their goal. Nevertheless, three European honey bee populations (Norway, France and Sweden) have survived for >10 years without mite treatments. It seems as if these bees have found a way to interfere with mite reproduction. However, it is not clear to what extent these adaptations are genetic or whether they are specific to the location these populations arose. Thus, it is crucial to know if these bees retain their ability to survive mite infestations when transported to a different environment. To answer this question, we will conduct a ring test across Europe. We will import queens from the three populations and test whether the bees are able to survive without treatment in different environments. We will also investigate mite and bee biology to understand why colonies survive or die. Given that the bees are also able to survive without treatment in their new environments, strong genetics are in place and future breeding should use them. Ideally, these populations can be used to identify genes responsible for varroa resistance. If, however, the bees will not survive, then local bees should always be used for selection programs.

16. Towards a Resilient Bee

Johannes Wirz

I would like to draw attention to a number of facts that – at first sight – do not seem to offer the potential to resolve the problems faced by the honey bees. We know from many studies that colony size and colony density strongly correlate with bee health. There are first guesses about possible mechanisms – colonies in France have chosen different strategies to tolerate the Varroa mite from to those in Sweden. Feral colonies in the Arnot Forest in the USA studied by Tom Seeley are surviving, having gone through a genetic bottleneck, but when transferred to his research apiary they were equally prone to fail like all the other colonies there.

On the other hand, initial studies investigating the expression pattern of genes, e.g. in bees fed with either sugar or honey, have unraveled conspicuous changes and allowed for identification of some of the genes' functions. It is likely that these expression patterns reflect the epigenetic status.

I would like to suggest making a thorough analysis of colonies that survive without any treatment (at least in six different regions in Europe and the US), not only of gene expression patterns but also of the contextual, environmental and beekeeping practice conditions.

17. What future for local bees in Britain?

Norman L Carreck[1,2]
[1]International Bee Research Association, Hendal House, Hendal Hill, Groombridge, East Sussex, TN3 9NT, UK. [2]Laboratory of Apiculture and Social Insects, School of Life Sciences, University of Sussex, Falmer, Brighton, East Sussex, BN1 9QG, UK.

The UK has never had a well-established queen rearing industry, so most colonies are headed by queens of unknown origin, but this has allowed near-native bees to remain in many parts of the country. Nevertheless, a number of firms sell imported queens and a number of beekeepers champion their use. The views of a number of professional conservationists and ecologists who claim that the honey bee is alien to Britain, and that honey bees have only a minor role in pollination have also been very unhelpful. A number of recent papers have, however, drawn attention to the possible disease risks associated with imported bees. The UK government's new National Pollinator Strategy covers all species of insect pollinator, but may provide opportunities for promoting the conservation of honey bees. The results of the recent COLOSS Honey bee Genotype-Environmental Interactions experiment published in the *Journal of Apicultural Research*, which show that locally adapted strains of bee consistently tend to perform better than imported strains provides support for the use of local bees over imported strains, and will hopefully encourage the further development of breeding groups working with locally adapted bees.

18. How to measure resilience? The immune system as focal point of honey bee health

Annely Brandt, Marina Meixner, Ralph Büchler
Landesbetrieb Landwirtschaft Hessen, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany
E mail: annely.brandt@llh.hessen.de

For successful beekeeping we need healthy and resilient honey bee colonies. The main threat to honey bee colonies is assumed to be the parasitic varroa mite that not only feeds on the bees, but also transmits a number of harmful diseases. For the health of the honey bees, it is crucial to have strong defence mechanisms against those parasitic infections and diseases. This defence can be weakened by environmental factors like pesticides or poor nutrition, which may leave the bees more vulnerable for parasites and pathogens.

We investigated the sublethal effects of the neonicotinoids thiacloprid, imidacloprid, and clothianidin on individual immunity in worker bees by studying total hemocyte number, wound healing, fat body content, immune relevant genes, and antimicrobial activity of the hemolymph. In laboratory experiments, we found a strong impact of all three neonicotinoids even at field realistic concentrations. These results suggest that neonicotinoids affect the individual immunocompetence of honey bees, possibly leading to an impaired disease resistance capacity.

Our data will contribute to a better understanding of the complex and multi-causal colony losses and may help to establish indicators for the health status of individuals or colonies as a way to measure resilience in honey bees.

19. Selection of acaricide resistance in Varroa and the need for resilient honey bees.

Robin F.A. Moritz
Institute of Biology, Martin-Luther-University Halle-Wittenberg

The swift selection of resistance to acaricide treatmenst severly affects the effectiveness of various acaricides. The use of these compounds is therefore a nonsustainable strategy to fight Varroa. I here highlight the population genetic mechanisms that greatly facilitate rapid selection in the mites. The population dynamics of the honey bee colony over the season in combination with the reproductive life cycle of the mite provide an ideal setting to allow recessive alleles for resistance to become quickly fixed in the population. Varroa undergoes several generations of strong inbreeding before heterozygosity can be restored at the end of the season. The strong inbreeding in combination with selection sets the stage for the rapid establishment of resistant mite lineages.

Appendix 3. Summary of the workshop discussions

What challenges are ahead? – small workshop discussion groups

In order to get an overview of the expected challenges – or call them knowledge gaps/important topics to work on – and to avoid duplication of research, we constructed an outlook of the challenges ahead of us. During the workshop, we asked the participants to more accurately describe their group topic (to check our conceptual frame work), to define sub topics if required, the key players, and most importantly the short-term (until 2020), mid-term (until 2025) and long-term (beyond 2025) challenges (Figure A3.1). For the group members see Appendix 1, where different colours show different groups; *orange* is called *Environment* (Table A3.2), green is called *Natural selection* (Table A3.3), blue is called *Breeding Programs* (Table A3.4), and. We asked the groups to fill in a prepared table (below) and present the outcome in a plenary session following the discussion in smaller groups.

Figure A3.1. Group discussion for Breeding Programs (left) and Environment (Right)

Table A3.1 Table that the discussion group were asked to fill in during the workshop

Main topic	**subtopics**	**Key players (to be) involved**	**Short-term challenge**	**Mid-term challenge**	**Long-term challenge**
Describe how the topic is defined/ interpreted by the group. Is there consensus?	*Define subtopics (use row for each subtopic)*	*Work shop participants* *Non-workshop participants*	*What are the main topics/ challenges that should be addressed until 2020?*	*What are the main topics/ challenges that should be addressed until 2025?*	*What are the main topics/ challenges that should be addressed beyond 2025*
Natural selection +Biology					
Breeding programs					
Environment					

TableA3.2 Environment					
Main topic	**subtopics**	**Key players (to be) involved**	**Short-term challenge**	**Mid-term challenge**	**Long-term challenge**
Environment Bees (including managed and wild bees, social and solitary bees) are subject to numerous pressures in the modern world: exposure to cocktails of agrochemicals, various pathogens, lack of abundance and diversity of feed, flowers and climate possibly incl. change. Stressors inevitably act in combination (*V. destructor* and X). Socio-economic factors (incl. globalisation) and human behaviour incl. beekeeper compliance and **steak holder interests** (e.g. consumer preferences, veterinary practices, farmer choices,…). **There are no two locations which share a combination of environmental factors. Each site is unique. In contrast to other livestock, bees are intimately interwoven into ecosystems. Therefore, breeding programs should take account of genotype-by-environment interactions** and be protected in time and space.	No good to adequately address the issue by thinking in boxes.	Norman Carreck Johannes Wirz Annely Brandt Robin FA Moritz Peter Neumann	**Adequate protection of resilient local bee programs by policy.** **Invasive species**	**Develop and implement bottom up approach from the beekeeper to the scientist.** **Science can only post hoc understand mechanisms causing local resilience. We should not select in all environments single traits, which are believed to be understood scientifically.** **Invasive species**	CCC (Climate Change Challenge) to address CCC (Colony Collapse Challenge). Globalisation in particular Asian beekeeping with *Apis mellifera*. **Invasive species**

Table A3.3 Natural selection and Biology					
Main topic	**subtopics**	**Key players (to be) involved**	**Short-term challenge**	**Mid-term challenge**	**Long-term challenge**
What is resilience? • overwinters well • good popula-tion growth • produce honey Resilience allows less treatments?	Host-parasite relationship: • *V. destructor* Resilient traits • Colony strength • VSH • SMR: Reproductive/non-reproductive mites	Funding agencies Specific experts outside beekeeping world	Understand mechanisms & trade-offs: • Genes Precise trait measurements: • Standardize trait measurements	Implement knowledge/breed for improvement: • Selection protocols Define key factors	Dissemination Instructions for management Easy assay/chip to determine trait values Better predictability of host-parasite relationship (model)

	General resilience traits:
	• Commonalities in resistant populations: SMR?
	• Biological resilience
	• Beekeeping resilience:
	  o need intervention?
	  o Trade-offs (honey)
		Funding agencies
		
		Specific experts outside beekeeping world
			Clear definition of tolerance and resistance:
			• Tolerance: cope with the stress
			• Resistance: reduce the stress load
				Find out needs from beekeepers
				
				Changing objectives from beekeepers (from honey to resilience)
					Changing objectives from beekeepers

	V. destructor biology (understand the variation in *V. destructor* infestations)	Funding agencies Specific experts outside beekeeping world	Compare different experiments (breeding/natural pops) on a large scale (big and detailed) Generate knowledge: • Population dynamics • Influence of environment • Reproduction cycle • Population genetics/structure • Mating biology • Mites in resilient populations	Define key factors Implement knowledge	
	Beekeeper biology	Beekeeping associations Professional beekeepers Funding agencies	Education/dissemination/extension Align beekeeping with bee biology: • Limited by situation Optimize beekeeping systems for local use in combination with selective breeding: • find easy traits • find markers for selection		

Table A3.4 Breeding Programs

Main topic	Subtopics	Key players (to be) involved	Short-term challenge	Mid-term challenge	Long-term challenge
Organization: Breeding structures to be enforced and supported for breeding resilient bees. Tools: Tailored breeding and selection tools to be developed and be made available to beekeepers.	Support local breeding structures to allow implementation of local and global developed solutions. Encourage real cooperation between beekeepers. Also important to multiplication/distribution of solutions.		x	x	X
	Develop resilient bees in close cooperation with research and beekeepers to ensure balanced traits.		x	x	X
	Molecular methods keeps developing and can strongly facilitate breeding.		x	x	X
	Propose breeding tools like databases, cryopreservation, practical, standardized, selection tools and protocols.		x		
	In steps towards *V. destructor* control without treatment. Integrated pest management (threshold based, including resistance management) for the intermediate term.		x	x	X
	Develop different (standardized) tools, which might best meet local and/or level of breeding experience.		x	x	X
	Support initiatives where measurement of *V. destructor* infestation is linked to propagation (leading to "genetic death" of *V. destructor*-sensitive genotypes).		x	x	X
	Exchange experiences in breeding to design optimal local solutions.		x		
	Local versus non-local bees are local decisions.				
	Treatment developments/application strategies needed for many years to come.		X	X	
	Support mating control (mating stations, instrumental insemination) to allow development of alternative and local solutions.		x	X	X
	Increase our understanding of relevant viral and bacterial diseases at low *V. destructor* infestation. Selection for stronger antiviral response might support overall resilience. [no consensus]				x

TOWARDS RESILIENT HONEY BEES – PLENARY WORKSHOP DISCUSSION

Some very exhilarating discussions took place between the groups. It seems that we can distinguish between two approaches that lead to resilient honey bees. The characteristics and conditions that define these two main approaches differ considerably, but there are also some (partial) similarities (and parallels).

Based on the plenary discussions on both days we made an overview of the characteristics and conditions that define these two main approaches (Table A3.5). Where do they differ, but also where do they meet?

Table A3.5. Overview of the characteristics and conditions that define these two main approaches. The more you go down in the table the smaller the differences become.

Breeding programs	**Natural selection and Biology**
Relatively labour-intensive process to gain a large population of resilient bees, unless an infrastructure of multiplication of improved material is in place. In special cases progress can be fast when using single-drone instrumental insemination.	Relatively low-labour method to gain a large population of resilient bees, but bottle neck and period with reduced numbers of colonies. More complex concepts may reduce the losses within the bottleneck
Selection on colony traits measured by the breeder (i.e. mite population growth, hygienic behaviour, VSH, SMR in addition to traits like gentleness and honey yield). Selection can be enhanced if queens can be effectively genotyped for markers or genes.	Selection on colony traits (i.e. survival, colony growth, reproduction)
Breeder (partly) chooses the mechanism for the bees to reach goal (autumn selection plus beekeeper trait selection included as a holistic 'practical resilient bee' approach)	Breeder lets nature (host and/or parasite) decide the mechanism to reach the goal (live-and-let die) within the limits of the particular population
Existing phenotype remains largely preserved, except for selected traits	Phenotype may change drastically
IPM required for some time (thresholds for treatments needed per region! Thresholds vary, depending on the virulence of the viruses in the colonies)	No treatment necessary
High honey production from start	High honey production can be obtained in additional phases. Low productivity could also be part of the end result. Beekeepers should then adapt beekeeping methods.
Bees adapt to beekeeper (balanced traits)	Beekeepers adapt to bees
Essential tools are already in place. Further improvement is possible by advanced technologies like marker supported selection and cryopreservation of semen and embryos.	Success depends on simple concept.
The body of knowledge on breeding is enormous and is equivalent to breeding of i.e. farm animals and pets.	Chances are that new ways to keep bees need to be developed and implemented.
Breeding structure & protocol can be used for new invasive species, but new key traits may have to be determined in case of extreme genotype x environmental interaction.	Exactly the same concept can be applied to new invasive species
Beekeepers provide breeders with wish-list or join in programs	Large commitment from beekeepers is required. They do the main work: temporary sacrifice of colonies and honey harvest and change the way to keep bees
Low risk for colony losses	High risk for colony losses, especially during bottleneck (but can be reduced by adapted approach). Losses may increase pest pressure in surrounding apiaries if no mitigating measures are implemented.

Breeding programs	Natural selection and Biology
Regional scale, but no scale limits	Local scale required
Adaptation to environment needs consideration in the breeding program. Some programs already do so.	Additional selection for adaptation to local environment is part of approach
Resistance	Resistance and/or tolerance
Pest will go to low levels of infestation (in case of *V. destructor*)	Stressors will not go completely extinct due to tolerance mechanisms
Genetic diversity within breeds will decrease (the amount you lose depends on the quality of the breeder and the amount and diversity of breeding material, number of drones mated per queen and on the diversity of breeding programs)	Genetic diversity within breeds will decrease (the amount you lose depends on the quality of the (group of) beekeepers , the size of the starting population and the intensity of the bottleneck
Breeding stations needed which are completely isolated	Breeding stations needed which are at least partly isolated
Process should be driven by beekeepers	
Two approaches are not mutually exclusive, provided that their breeding stations are kept separate	
Understanding the mechanisms (and traits) causing resilience is important to the management of the resistant colonies, as it helps to identify potential trade-offs and give insight in the colony biology and i.e. the parasite-host relationship	
Balance between fundamental and practical research is needed	

Note: the interpretation of 'local adaption' seems to differ between the two lines. 'Breeding' tends to refer to small networks of beekeepers that start local breeding programs and organize their local breeding infrastructure resulting in locally adapted managed colonies (specific race or not). Whereas, 'Natural selection' tends to refer to natural adaptation of the bee colonies to the specific local environmental conditions. i.e. soil types and related vegetation, climate

Although Resilient Honey bees should ideally be resilient to the widest variety of pests and diseases possible, these bee colonies will never be able to cover the whole spectrum at once. There was major consensus on *V. destructor* being the most important pest to tackle at this moment. But it was noted that what is good for *V. destructor* resistance may facilitate other diseases that need other conditions. Many diseases are however vectored by *V. destructor* and are expected to reduce with increased *V. destructor* resistance. At the same time efforts should be made to reduce as much as possible bee exposure to unnecessary stressors (i.e. agricultural stressors or beekeeping stressors that originate from mismanaged or misuse).

To produce resilient bees for beekeepers most of the breeding tools we need are already in place, although technologies to further increase genetic progress are highly desirable. The knowledge gaps in other aspects (natural selection, biology, and the environmental aspects) however need further refining. For all areas implementation efforts are expected to be major still. 'Implementation will take 15 years, even if we know everything tomorrow', was one of the remarks during the workshop.

BOX: Are transgenic Honey bees a future solution for *V. destructor* resistance?

We discussed this topic during the workshop. Believe this question will arise in the future, but this is an area in full development and politically sensitive. Current developments do not seem far enough yet to decide now if transgenic honey bees resistant of *V. destructor* may be a valid future solution. One major hurdle we see now is the free foraging of honey bees in the field, which cannot be confined and raises questions on how to deal with intellectual property. Regulation of ownership should be organised and political strategies on how to deal with these issues defined. Impacts for environments should be determined.

For the balance between fundamental and applied research, there are some questions that need more clarity:

- How does the pathogen (pest or disease) affect the colony as a whole?
- How can a colony become resistant?
- How to involve beekeepers? Their commitment is essential in all approaches and they are key stakeholders in the process to gain resilient honey bees

Some ideas and existing initiatives to involve beekeepers have been shared.

For the breeding approach:

- Invite experienced beekeepers to participate in breeding programs such as AGT (abstract 10), Arista Bees (abstract 11), Duurzame bij (abstract 13), or beebreed.
- Offer services and get something back (perhaps: measure resistance of colonies?)
- Make standard protocols for breeding and training of breeders
- Use monitoring programs to make an inventory of the wishes of the beekeepers. In Germany and other countries standard monitoring was extended by questions like 'What are your needs with respect to education and/or beekeeping?'
- Standardizing of methods was done already in the COLOSS BEEBOOK (i.e. breeding, including measurement of traits and rearing) + beebook platform. Beebook is however for scientists. Within the EU SmartBees project scientists are working on a master version of a 'beekeeper/breeder beebook' for breeding (translating the Beebook breeding chapters into a beekeeper-friendly booklet), which is expected in 2017. Also, the book '*V. destructor*, still a problem in the 21st century?' explains the ins and outs of breeding for beekeepers.
- Workshops with breeders to implement and locally adapt breeding protocols. This could be an example for breeding structure.
- Internet platform (per country) to get knowledge to beekeepers about standardisation of breeding strategies

For the natural selection approach:

- Facilitate initiatives such as VBBN Laren (abstract 7 appendix 2)
- Use experience Bees@wur with the *V. destructor* brochure to develop a sort of cookbook for the natural selection concept
- Internet platform to motivate and inspire beekeepers to form initiatives to stop treatment and start selecting for resilient honey bees.
- Involve beekeepers in science projects (for example CSI pollen within the COLOSS network; www.coloss.org). Internet platform (per country) to get knowledge to beekeepers about the need and methods of locally organized natural *V. destructor* resistance selection.