Minutes of the
3rd COLOSS conference
Belfast, UK, 6.-7.09.2008

1. Organisational matters

Steering committee
After the retirement of Agnes Rortais, new members were obviously required. Rob Paxton suggested Asli Özkirim and James D Ellis, who were both approved by the general assembly and accepted the duty.

Next meetings
It was decided to organise the next meeting in Istanbul between the end of February and beginning of March. A respective doodle has been launched, revealing the 3.-4.03.2009 timeframe as being most appropriate for the majority of the members. Further details will be communicated after the meeting in Brussels (21.11.), as some points need to be clarified. Since COST support will only be possible for meetings in COST member countries, it was decided to relocate the Spring Meeting 2010 from Egypt to Croatia, where Nicola Kezic kindly offered to do the local organisation.

2. WG matters

WG 1: Monitoring and Diagnosis

Participants: Nizar H., Kristiansen Pr., Flemming V., Gjessing T., Brown M., Charrière J.D., Mutinelli Fr., Ozkirim A., Moosbeckhofer R., Ritter W., Le Conte Y., Dauzet Ph., Topolska G., Santrač V., Lee S.

A – MONITORING

I- Development of standardized monitoring
1. Questionnaire:

Romée van der Zee presented this topic, continuing, and including responses, from the previous meeting in Athens.
Internationalization and climate classification was discussed. Romée will send information and examples of climate classification so that we can work on it and discuss for the next meeting. For example, zip code can give information about local differences.
2. Losses and surviving colonies:

Winter losses were discussed and the discussion focussed on the necessity to include weak colonies to losses. Do the losses include weak colonies? Is a weak colony a handful of bees? How to quantify this?
We agreed to give propositions to Romée on this topic in the next 5 weeks.
3. Definition of CCD:

We argued on the definition of CCD and agreed that it should be more precise and better named.
4. First step: building up a simple questionnaire.

We stressed that we should exchange information between the meetings and interact on this topic. A questionnaire will be send to us by Romée, so that we will be able to

work on it and finish it early 2009. We decided to build up a simple questionnaire including simple questions to estimate the mortality in the different countries. It will be on internet and for the different countries and beekeepers. A chosen phenologic state could be chosen, as for example dandelion blooming, to start the monitoring and fill up the questionnaire. This would avoid the problem of introducing weak colonies, as at this period (dandelion blooming) those weak colonies should be dead (or hopefully alive). This internet questionnaire could be filled up real time. Local representatives of beekeepers could have access to the questionnaire.
This work could be supported financially by our COLOSS action. If so, it would be our first action inside the group. Romée proposed to manage this project as pilot. It could be first tested inside our group and then proposed internationally.

An other questionnaire, including more information, could be done for research purposes.

II – Monitoring for colony losses

Aim: find the causes!

Vejnaes Flemming gave an interesting talk about what he is doing on this topic, as a basis of our discussion.
Monitoring can be used for extension purposes to find a solution or for scientific purposes to look more in details.
We distinguished three different levels:

- Questionnaire level to estimate mortality or disease problems. Is a tool to detect problems in an open group.
- Evaluation level to try to find the causes of problems, using diagnostic.
- Monitoring level to follow up a group of colonies for a long period

An Example (debimo) given by Wolfgang Ritter: 125 beekeepers with 70.000 colonies in all different regions of Germany and all types of beekeepers, using a protocol filled up with advisors, samples taken from 10 colonies per yard. Is helpful for diagnostic but very expensive (paid by different parties). But in our COLOSS approach, we can use existing system and network. Monitoring can also tell us how we have colonies which survive.

II - 1 – Monitoring for extension services.

It should be a basic monitoring including different parameters.
Diseases:

- Varroa
- Nosema (PCR not necessary)
- Acarapis woodi
- Virus not needed because there is no treatment to control them, but we can look at deformed wing bees.
- AFB, EFB
- Chalkbrood
- Sacbrood: we can include it if there is a beekeeper needs.

Questions on management (food supply, size of the box for overwintering…), environment including plant protection and pesticide problems must be left open. Vejnaes Flemming will work on this issue for the next meeting. We can send him questions and ideas.
We then worked on two questions:

Is it necessary to follow the same beekeepers and the same colonies?
We think that beekeeper level is acceptable, not the colony level.

How many time should we check the colonies per year?
We concluded that twice a year, in spring and autumn is the minimum.

We can also recommended to send our monitoring protocols as examples to Vejnaes. It will be helpful for him to work on a standardized one for the general purpose.

To summarize: Monitoring for extension must be quick, easy, cheap and effective!

II - 1 – Scientific monitoring.

Kim Nguyen Bach and Haddad Nizar agreed to be in charge of this topic. Other member of the COLOSS group can send them examples of scientific monitoring to help them.

B - DIAGNOSTIC

There are needs in exchange of knowledge and training courses in this topic. It should be a second action developed and financially granted in the framework of our COLOSS action.

There are also needs to standardize techniques (for example to quantify Nosema spores). Ring test could be done for some diseases.
Wolfgang proposed to give the standard operation procedures and so we can work on it.
For quantitative methods, quantitative PCR is needed.
We also need protocols to establish the level of varroa mite.

Yves will collect data on new techniques for diagnostic tools. If you have material to sent to him, it will be useful for this synthesis.
Wolfgang will collect data on references techniques for diagnostic.
Reference material could circulate though COLOSS.

C – FINAL DISCUSSION

Romée ask to set up a small committee to work with her more closely to establish the questionnaire and protocol for early 2009.

Demand of all of you about:
- Protocole for extention monitoring
- Protocole for scientific monitoring
- Protocole for varroa infestation

WG 2: Pests and Pathogens

Participants:

Member	Focus (in this network)
Rob Paxton (UK)	Viruses & Nosema
Joachim de Miranda (UK, Sweden)	Viruses
Nor Chejanovsky (Israel)	Viruses
Dalibor Titera (Czech Republic)	Varroa, AFB, Nosema, reference lab
Maria Navajas (France)	Varroa, genetics, **link to WG 4**
Raquel Martin Hernandez, Mariano Higes, (Spain)	Nosema, Varroa, **link to WG 1**
Annette Schuermann (Germany)	treatments (Nosema, Varroa)
Prof. Emmanouli (Greece)	GMO, pesticides & Nosema
Zlatko Tomljanovic (Croatia)	Extension specialist, **link to WG1**
Antonio Felicioli (Italy)	Biochemistry, proteomic, parasitoid fly
Gianluigi Bressan (Italy)	Varroa, extension specialist, Mediterranean
James D Ellis (USA)	SHB, Varroa, viruses, pesticides
Peter Neumann (Switzerland)	SHB, viruses, Varroa, bacteria, **link to WG 4**
Marika Harz (Germany)	Varroa control, toxicology, pharmacology
Tjeerd Blacquière (Netherlands)	Varroa interactions with other pests, **link to WG3**
Antonio Nanetti (Italy)	Varroa, Nosema, parasitoid fly

Topics of Discussion

1) Organisation matters:
A number of suggestions were made:

A) WG links: within each WG people should be responsible for linkage to other WGs.

B) Call for collaborations: Mycologist, physiologist

C) COLOSS website: Protocols, chat rooms, news site with abstracts of papers being in press? etc.

D) Skype phone conferences?

E) WG meetings (work shops) On more specific subjects (e.g. Nosema, or diagnosis) before or after general meetings?

F) Corporate COLOSS identity (e.g. for recommendations to beekeepers)
In each COLOSS country (**COST deliverable**)

2) **Themes:**
It was decided to focus in WG 2 on interactions between pests and pathogens, which are Inevitable due to the ubiquitous mite *V. destructor*. There are however, obvious links between WG 2, 3 and 4.

Factors, which also influence disease progress both in individual bees and in colonies were discussed. Among those were sub lethal dosages of pesticides, pollen quality/quantity, genetic background & beekeeping techniques, weather, apiary (almost everything, Varroa might predispose for poisoning or vice versa?). We decided not to classify factors, because we are not aware of the underlying factors (hen and egg dilemma). With respect to experimental approaches, factors were nevertheless grouped according to possible control measures.
A) Control possible (e.g. genetic background, beekeeping techniques)
B) Control impossible (e.g. weather)
c) Geographical constraints (e.g. Varroa & viruses are impossible to completely control in some areas)

How to find consensus about methods & common experimental procedures?
It was decided to develop / decide about common COLOSS protocols ("best practice") both in terms of Diagnosis (OIE, COLOSS & national reference labs) as well as Experiments (e.g. for hoarding cages). Dissemination of COLOSS protocols will be performed via website (http://www.coloss.org) and will most likely be a suitable **COST deliverable**.

Some suggestions were made for common experimental approaches (see below).

COLONY LOSSES AND PATHOGEN INTERACTIONS SUGGESTIONS FOR EXPERIMENTAL APPROACHES

Ingemar Fries[1], Antonio Nanetti[2], Peter Neumann[3]

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[3]Swiss Bee Research Centre, Agroscope Liebefeld-Posieux, Research Station ALP, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland

Suggestions are based on recommendations by the Pathogens subgroup of the COST project COLOSS at the 2nd project meeting in Athens, April 2-3, 2008.

Colony losses and pathogen interactions

Background

Colony losses have been occurring recently at higher frequency, with higher magnitude and also exhibiting different symptoms (CCD = Colony Collapse Disorder). Undoubtedly, the introduced external parasite *Varroa destructor* has caused massive colony mortality world-wide, but this parasite alone cannot explain the major losses now experienced in large parts of the world. Some other already established pathogens (e.g. bacteria, viruses) and environmental factors (e.g. agrochemicals, malnutrition and beekeeping management) may also cause colony losses and have been implicated as major drivers for colony mortality. Insufficient beekeeping and/or intensive exploitation of honeybee colonies for crop pollination and honey production are also likely to play a role for colony survival. Moreover, environmental and climatic changes in terms of intensifying agriculture monoculture, warming up in continental climatic regions and electric fields have also been discussed. Further complicating the picture are novel factors, e.g. GMO or the introduction of new pests (e.g. small hive beetle, microsporidian parasite *Nosema ceranae*). However, the relative importance of these potential factors in the recent major losses is simply unknown, due to an apparent lack of reliable and comparable field data on losses. Furthermore, state-of-the-art standards in honeybee diagnosis are lacking (e.g. standardised metagenomic surveys), despite recent advances in DNA/RNA methods and the sequenced honeybee genome. Even more important, synergistic interactions between factors contributing to colony losses are only poorly understood, e.g. between pathogens (*V. destructor* and viruses) or between sub lethal effects of pesticides and immune response. Impact of the former has severely been underestimated, because multiple infections with pathogens are inevitable due to the ubiquitous mite (*V. destructor* + x), with global trade a contributory factor. Finally, human selection and breeding of European honeybee subspecies has resulted in calm, manageable and productive colonies, but may have undesired side effects of reduced genetic diversity of local honeybee populations. Reduced genetic diversity may foster further losses due to the vulnerability of bees to inbreeding (sex determining mechanism of Hymenoptera) and due to reduced adaptations of bees to local and global influences as well as to different diseases and parasites.

Protocols for studying pathogen interactions

Earlier attempts within European networks for standardizing protocols for specific investigations have been largely successful. A good example is the Concerted Action FAIR CT97-3686 “Coordination in Europe of integrated control of *Varroa* mites in honey bee colonies” where evaluations for efficacy of different *Varroa* control methods was standardised. To make investigations comparable between laboratories, it is undoubtedly of great value to use standardised protocols. However, FAIR CT97-3686 succeeded in developing and implementing protocols for a specific defined purpose. To develop a universal protocol aimed to cover interactions between all available pathogens and all possible factors of interest is simply not possible, largely due to the variation in the various host-pathogen relationships. At best, we can attempt to formulate a more general framework, within which we should attempt to develop specific protocols for the specific interactions of interest.

When pathogens interact within a host, the outcome may be very different compared to single infections/infestations. Even within host competition between different strains of the same pathogen may radically alter the level of virulence exposed by pathogens. The best known example from honey bees on the dramatic effect on virulence from interacting pathogens is the impact from virus infections on honey bee colony survival. As latent infections become overt, due to the feeding behaviour of the mite, and as the vector function of the mite radically alters the routes of virus transmission, the colony level virulence of this pathogen combination, widely surpasses the effects from mites or viruses as isolated phenomena. Obviously, if we want to understand colony losses, it is necessary to unravel how a large number of pathogens interact within the bee colony and within the individual bee, and how this outcome in turn is influenced by environmental and genetic components. Multifactorial causes of a given disease are difficult to study, because it is often difficult to determine if the experimental scenario created really represents all relevant factors. Therefore, traditional studies of honey bee pathogens have often attempted the opposite, to standardise conditions and study a specific pathogen in isolation. This standardised approach and studies of isolated pathogens is needed also for studies of pathogen interactions, but then as a contrast to when different factors or pathogens are combined. Thus, studies of interactions must be based on an approach where the level of complexity is increased step by step, and where each level of complexity is evaluated against lower levels of complexity.

Individual level and colony level interactions

Evaluating the impact of pathogens on honey bees requires that the system is studied at two levels. It is the individual bee or larva that contracts a disease, but it is the colony level impact from individuals being diseased, that eventually may cause colony collapse. And this collapse may be caused by specific disease, interacting pathogens and/or in combination with predisposing factors that may influence disease progress, both in individual bees and in their colony.

In general terms, laboratory investigations using caged honey bees (of defined age and background) should be the first step for investigating pathogen interactions. The field situation using intact colonies will always produce more variation and influence from factors out of control and the use of full sized colonies is costly, in particular if colonies need to be sacrificed. Thus, the most logical approach is to investigate interactions at the individual bee level, and where interesting results are produced, take this specific topic further for field testing. In Figure 1, we outline the experiments

necessary to evaluate the interaction between two pathogens and one external imaginary factor at the individual bee level.

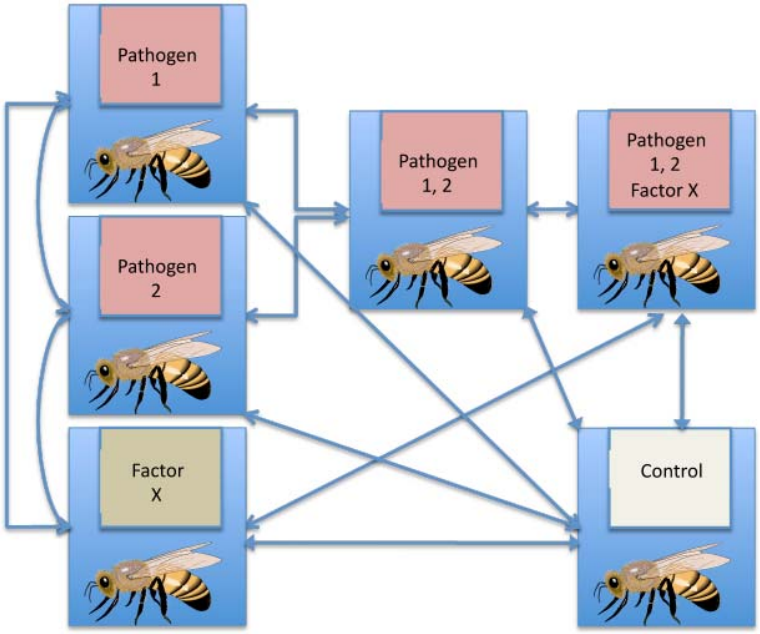

Figure 1: *Schematic representation of experiments needed to evaluate the effects from interactions between pathogens within individual bees. The individual bee level effects are likely best investigated in cage experiments. Repetitions of cages are needed because there is often a cage effect. Each box represents different experiments and the arrows represent comparisons between experiments to evaluate the impact from the addition of further complexity by introducing pathogen interactions, and/or interactions with defined factors.*

Individual bee investigations should include the following registrations:

Pathogen/Factor parameters
Growth rate of the pathogen(s)
Infective dose of the pathogen(s)

Host parameters
Mortality rate/Longevity
Pathogenic effect

Pathogen interaction regarding brood pathogens should follow the same structure as outlined for individual bee studies (Figure 1). When young brood (before sealing) is studied, the protocol for *in vitro* larval rearing (appendix 1) can be used. When older brood is studied, the same protocol can be used, possibly supplemented with incubation of sealed brood in incubators, using brood temperature (+34 ºC) when appropriate (i.e. when *Varroa* mites and interactions with that parasite) is studied.

Individual bee investigations form a foundation for determining what may be interesting to look at, at colony level.

Colony level investigations

Colony level investigations cannot, in many cases, be conducted as a replicate of lab experiments. Nevertheless, wherever possible, the same basic structure for comparisons should be followed (Figure 2).

Colony level investigations should include the following registrations:

Pathogen/Factor parameters
Pathogen prevalence and rate of infection

Host parameters
Bee population dynamics (brood and adult bees, i.e. Liebefeld method)
Bee mortality (bee traps)
Colony mortality
Productivity

It may not be possible to add or remove pathogens in field experiments similar to cage experiments. Field experiments must be based on sufficient number of colonies because variation is likely to be greater in the field. Control colonies are another problem. To avoid infection of control colonies they are best situated in another isolated apiary. However, then you may have an apiary effect not controlled for. Each specific comparison or experiment may be different regarding these considerations. If control colonies are used in proximity of infected colonies, suitable measures (i.e. spacing and entrance directions) should minimise drifting between colonies.

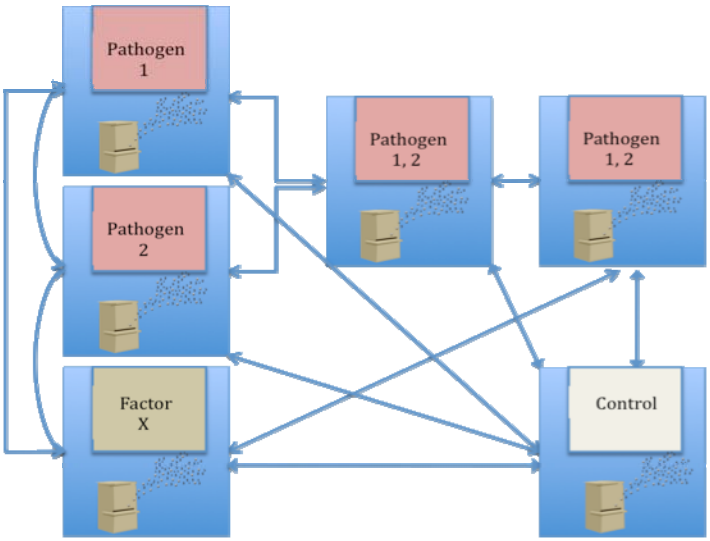

Figure 2: *Schematic representation of experiments needed to evaluate the effects from interactions between pathogens within colonies of bees. Each box represents different experiments and the arrows represent comparisons between experiments to evaluate the impact from the addition of further complexity by introducing pathogen interactions, and/or interactions with defined factors.*

WG3: Environment & Beekeeping

Coordinators: Karl Crailsheim & Aleš Gregorc

Participants: Bouga Maria, Crailsheim Karl, Gregorc Ales, J. van der Steen, T. Blacquière, Chauzat Marie-Pierre, Vaissiere Bernard, Koltowski Zbigniew, Dalibor Titera.

WG evening discussion (September 6th): Karl Crailsheim welcomed the participants of the WG3 meeting and gave a short introduction the activity proposed in the work plan for Belfast 2008 meeting:

Nutrition: contamination and beekeeping praxis, nutrition-related questions, influence of environmental conditions, environmental pollution, available pollen sources will be studied on larvae and adults level.
Intoxication: Environmental pollution, pesticides and their influence on adults and larvae; organs, tissues. Studies of physiological, developmental ability.
Hive management: diverse beekeeping management, different hive types, technology, queen rearing. Studies on individual and colony level including aspects of physiology, vitality, intoxication, genetics.
Genetic impacts: synergy between pathogens, bee breeding, selection, environmental stressors.

Present members of the WG 3 introduced their research work and interests:
Vaissiere Bernard: studying pollination, landscape.
Koltowski Zbigniew: pollination, beekeeping value plants
Chauzat Marie-Pierre: bee biology, pathology, ecotoxicology, cellular markers for stress.
Titera Dalibor: insemination and selection, bee breeding
J. van der Steen, T. Blacquière: ecotoxicology
Bouga Maria: bee projects - DNA damage (comet assay) cause by different influences
Gregorc Ales: sublethal effects caused by different influences, cell death and cell stress localisation, pathogens and interactions.
Crailsheim Karl: American foulbrood, larval and thermal behaviour, electromagnetic fields and honeybee nutritional requirements.

Conclusions of the WG3:
- members of WG3 are currently performing and adopting research methods and their research is conducted on material originated and/or supplied from members labs.
- uniform material (larvae, adult, specific races bees), derived and spread from specific Labs (example: LAB A: for reared bee larvae; LAB B: for specific bee race – black bee, carniolan etc.), and research techniques are also offered to different research groups.
Same lab – lab A can play different roles: as a donator of material and/or as acceptor of material and conducting research.

Possible scheme of Labs collaboration and material exchange:

LAB: A is a source of material (bees, larvae) LAB: B, C, D…: perform research

Bees Methods – treatments:
Treated bees
Treated colonies
Isolated organs and tissues: HPG, salivary glands, midgut… Pesticides
Different food
Pathogens,
Electromagnetic field influences
Combinations
Other stressors

Collaborations between labs:
- exchange material
- exchange – transfer research methods
- training courses
- international and national funding Stress evaluation, cell and molecular biology, pollination, genotyping :
- pollen sources: effect on individual bee or colony
- eco-toxicology
- Genotoxicity
- potential bee loss factors

Coordination of the work between different WGs:
Discussion on the second day (September 7th 2008) was organized between WG3 and WG4: members of both groups agreed in common research interests with emphasis in studying:
- vitality
- genotyping + environment: races – species + stress factors
- biodiversity: conservation strategy
- viability tests: technological aspects of keeping bees, diseases control and etc.

General conclusions:
- inter-laboratory collaboration in aspects of material and research techniques exchange as a priority in achieving the goals of WG 3 and also for the whole project.
- individual and/or organized training courses with priority between the members of WG3 members and other groups.
- collaboration and organized discussion between WGs.
- effective exploration of national funding sources for conducting research and possible international collaboration

Protocol: Aleš Gregorc **Coordinators:** Karl Crailsheim & Aleš Gregorc

WG 4: Diversity and Vitality

Participants: Bienkowska, Malgorzata; Bouga, Maria; Büchler, Ralph; Costa, Cecilia; Hatjina, Fani; Ivanova, Evgenija; Kence, Aykut; Kence, Meral; Kezic, Nikola; Kryger, Per; Meixner, Marina; Panasiuk, Beata; Uzunov, Aleksandar; Wei, Shi; ? Ahmed

Topics of Discussion

Our agenda consisted of the following topics:

1) Parameters of vitality
2) genotype-environment interactions
3) Biodiversity of honeybees in Europe
4) Joint discussion with WG3

For each of the discussion topics proposed in the agenda we discussed 1) defining the goals the Working Group aims to achieve by the end of the COST action, 2) which steps to take in the first year towards such goals, 3) which aspects the different research groups will focus on, and 4) which COST instruments can be used.

1) Parameters of vitality

A standard breeding protocol was developed at the 1972 Apimondia Symposium in Lunz, where clear criteria for the performance testing of colonies were defined for the first time. We now agree that our goal is to integrate vitality parameters into this commonly accepted protocol for colony evaluation during the COST action.
To this aim, it is first of all necessary to define criteria with which “vitality” can be measured and compared in field tests. Currently, there are projects underway in some countries that use different ways of measuring tolerance to stress factors, disease resistance and overwintering ability.
Therefore, as a first action, several group members (Cecilia, Fani, Malgorzata/Beata, Ralph/Marina, Nikola) will work together to summarize criteria of vitality that are currently being used in different breeding programs. All participants agreed to send their contribution to Cecilia Costa, Ralph Büchler or Marina Meixner by November 5. This will produce a preliminary protocol that will be discussed at the spring meeting. Evaluation and implementation of criteria will follow in a further step, possibly using the instrument of training schools.

2) Genotype-environment interactions

During and after the plenary session on colony losses, several observations were reported that local strains of bees apparently were less affected by losses than imported strains. At the same time, there are already some lines existing that apparently have better strategies to cope with Varroa than others. After discussing these points, we decided to collect and evaluate information from individual members on better survival of endemic races. This information can be published, unpublished or anecdotal. The participants agreed on sending relevant information and observations to Marina Meixner by December 5.

Apart from this activity, several members of WG 4 (working with different genotypes of bees) engage in large-scale routine field tests of colony performance. Thus, stock

queens could be exchanged between such groups to reveal interactions between genotype and environment (and/or test methods). This kind of comparative survival test in various locations will have to run for several years and require additional resources (coordination, exchange visits, meetings, PhD student?). Ralph Büchler agreed to coordinate such activities.
We also expect to use the results for the development of sustainable management strategies adapted to different regions of Europe.

3) Biodiversity of honeybees in Europe

Our goal is to consolidate the currently available knowledge on biodiversity of honey bees in Europe, since relevant information on the variability of honey bee populations is not always readily accessible. Given that locally adapted bees may be better able to cope with diseases, parasites and environmental stress factors, an inventory of the currently available knowledge, as a prerequisite of defining gaps and further research needs, appears ever more important. To this aim, we will start by producing a reference list, comprising publications and other references, sample collections and databases. Relevant information should be sent to Marina Meixner, Per Kryger or Maria Bouga. At the next spring meeting we will discuss how to proceed further.

4) Link to WG3 – joint discussion

We discussed the importance of defining a “health status” of individual bees. This could be useful for comparison in genotype-environment interactions and subsequently in breeding programs to define a healthy colony. As a first step, currently used parameters for “bee health” will be collected (Karl Crailsheim) and discussed at the spring conference.