Minutes of the
2nd COLOSS conference
Athens, Greece, 2.-4.04.2008

1. Organisational matters

Working group organisers

1) **Monitoring and diagnosis:** Yves Le Conte & Wolfgang Ritter
2) **Pests and Pathogens:** Ingemar Fries & Peter Neumann
3) **Environment and beekeeping:** Karl Crailsheim & Aleŝ Gregorc
4) **Diversity and vitality:** Marina Meixner & Cecilia Costa

Steering committee

Ingemar Fries suggested the following steering committee members, who were approved by the general assembly and accepted the duty:

1) Blacquière, Tjeerd
2) Crailsheim, Karl
3) Hatjina, Fani
4) Rortais, Agnès

Next meetings

It was decided to change the terminology into “conferences” rather than work shops. After kind suggestions by the local organizers, the next meetings will be as follows:

1) Autumn conference 2008: prior to EurBee in Belfast, UK, 6.-7.09.2008
2) Spring conference 2009: in Ankara, Turkey, organised by Aslı Özkırım
3) Autumn conference 2009: prior to or after Apimondia in Montpellier, France
4) Spring conference 2010: in Minia, Egypt organised by Hassan Adel Rushdy

2. Estimates of colony losses

In the different working groups, the participants reported n the bee and bee colony losses in their respective home countries. The data are shown in the following table:

Table 1:

Country	Losses	When	where
Germany	~10% > 30%	2005 to 2008 2007/08	Monitoring project in south
Austria	8 to 15%	2007-2008	depending of regions
Poland	20% >30%	2007/08 2007/08	from questionnaires from samples
USA	30% < 17%	08.07.2006 before	
Belgium	8 to 18%	2006-2007	
Croatia	16%	2005-6	
	25%	Winter 2007-8	
Finland	16%	98-02	
	34%	2002-03	
	10%	2003-07	
Italy	30-40% 10-30%	2007 2007	North South
Greece	5 to 25%	2007	survey (166 questionnaires) depending on the area
Switzerland	30%	2007-8	
	10-30 %	2002-7	
Denmark	15,9 22-25%	1986- 2006 2007-2008	
Netherlands	13-26%	2003-08	
Bulgaria	6%	2007	
Turkey	40% 10%	2007 2007	*Carnica* & *caucasica* *Anatolica*
Sweden	devastating	2002-3	
Slovenia	27% 20%	last winter 2007	

WG 1: Monitoring and Diagnosis

Participants:
Charriere, Jean-Daniel; Flemming, Vejnaes; Hatjina, Fani; Korpela, Seppo; Le Conte, Yves; Mutinelli, Franco; Nguyen Bach, Kim; Pettis, Jeff; Ritter, Wolfgang; Santrac, Violetta; Topolska, Grazyna; Trouiller, Jerome; van der Zee, Romee

In WG 1, like in the other WGs, the actual losses were presented. Afterwards, we discussed the following core issues:
- Development of a standardised monitoring
- Regional analysis
- Optimisation and standardisation of diagnosis
- Development of a prediction system

Development of standardized monitoring

The individual speeches referred also to the questionnaires on determination of the actual losses and the protocols for a monitoring to identify the reasons. These suggestions were discussed consecutively under the respective topics of the agenda.

In general, we agreed that the questionnaires and protocols for the extension service and scientific examination vary, among others, from their scope and their elaborateness.
Moreover, the question on anonymity of questionnaires and protocols was discussed. This issue has to be left to the individual countries because of their specific prevailing situations. In order to receive data as realistic as possible and answers really true, it may be helpful to have them given anonymously. On the other hand, the data of protocols can only be validated and can only be evaluated in chronological order if they are collected from defined bee yard or bee keeper. At any rate, for data protection reasons, a publication of these data can only be done in an anonymous way.
Notifiable diseases were another point of discussion. Here there were different opinions about the question if an outbreak of American Foulbrood could be part of questionnaires or protocols. The same is valid for the taking of samples for analysing these diseases. Therefore, we agreed to leave this also to the individual countries and to give no recommendation.

Questionnaires to register the actual losses

The questionnaires should facilitate to collect data about the actual losses. By this simple way of interrogation we can clarify within a relatively short time if there is a problem, i.e. any loss, at all. Questioning to receive reliable and certified statements needs a much bigger effort. For statistical reasons, at least 5% of the bee colonies or even better 5% of the beekeepers in a country should be involved. Doing this, factors like different climatic zones, foraging situations, bee density and management systems should be taken into account. Those data gathered from questionnaires urgently need to be validated. One possible way would be to collect the data on all losses with nearly all beekeepers in a strictly defined manageable area. Various suggestions for questionnaires were discussed to some extent. These suggestions respectively examples are to be sent to the responsible person in charge, Romee van der Zee. She will evaluate them concerning compliance as well as clarity and necessity of the questions. Moreover, she will submit the suggestions for validation of

the data. In our next meeting, we will report and present first proposals on possible questionnaires.

Protocols for monitoring First it was suggested to use the German monitoring protocol as basis for further discussions. The speeches and discussions, however, revealed that we have to distinguish between protocols for the extension service and those for scientific estimations. Therefore, the two sectors were separated and one responsible coordinator per sector was defined:
Flemming Vejsnaes (protocols for extension service)
Nguyen Bach Kim (protocols for scientific estimations)
Respective suggestions should be remitted to them to enable them to elaborate corresponding suggestions, as in case of the questionnaires, until the next meeting.
It was agreed upon that a monitoring only makes sense if one half of the participating apiaries either had problems or didn't have any problems with colony losses in the past. Only then possible solutions for problems as well as optimal ways of avoiding problems can be shown. It was also agreed that the monitoring scope in the individual countries depends essentially on the financial means available. Therefore, only recommendations can be given on this occasion.
So, also the question how often apiaries should be visited per year could not be answered definitely. One time per year is the minimal requirement, two times would be better at any rate. Part of the data can also be collected by phone or by correspondence. However, a direct contact to the beekeeper should always exist. However, the necessary efforts, apart of the financial means at hand, mainly depend on the purpose the data are collected for. The coordinators will submit corresponding suggestions.

Standardisation of diagnostic methods
The diagnostic methods used for the monitoring are essentially defined by the purpose of the study itself. If the examinations are part of the extension service the methods described in the OIE manual are sufficient. For scientific examinations, however, more specialised methods will have to be applied. For the individual sectors reference laboratories were chosen:
Freiburg (OIE methods)
Avignon, Beltsville (Scientific methods)
Reference laboratories are charged to organise training courses and to guarantee an exchange of methods and knowledge.

Development of a system for prediction of possible losses
The data of the regional examinations should be used to predict possible losses. This issue will be discussed during the coming meetings.

WG 2: Pests and Pathogens

The WG 2 discussions centred around three main topics

1. Interactions between pathogens with multiply infested/infected hosts
2. Environment and pests (temperature and *Varroa destructor*)
3. Pesticides and pests/invasive

Multiple infestations/infections

An interesting feature of honey bees infested by *Varroa* is that whatever other pathogen might be present, you have always the mite infestation plus something else. Multiple infestations/infections will therefore be the general focus of WG 2, but aspects of individual pathogens should also be considered (e.g. *Nosema apis* vs. *N. ceranae,* alone or in combination) in WG 2.

The changing effects of mite impact since the introduction of *V. destructor* into new areas (lower infestation levels tolerated over time) were recognised as a promising area of research to understand multiple infestations/infections. Anecdotal evidence propose that honey bee colonies can tolerate lower infestation levels after years of mite infestation, and it has been suggested that there is a link to increased general higher levels of background virus infections when the mite has been well established in the bee population. However, there is no hard data substantiating this hypothesis. Suitable areas to study this possible phenomenon should be areas where the mite has newly arrived or is presently being introduced. Such areas could include parts of Sweden, New Zealand and southern Africa. Possible topics for studies of multiple infestations/infections could include 1) prevalence and virulence of microorganisms associated with *V. destructor,* 2) *V. destructor* infestation levels themselves, 3) studies of the interaction between multiple infections of microorganisms (i.e. infectivity and virulence of bacterial disease in larvae in the presence of other pathogens or beneficial bacteria).

Environment and pests (temperature and *Varroa*)

Abiotic factors influence the honey bee population dynamics. As the climate changes, the honey bee population dynamic will change in specific geographic locations. It is important to better document the influence of climate on brood rearing dynamics to better project when colonies are at risk of producing damaging mite levels in different locations and climate types. Thus, brood measurements and bee estimates are important as background information for understanding mite population dynamics and should be more widely used. If the same basic technique is used (Liebefeld method) data can be comparable and a better general understanding for the effect of abiotic factors on mite population development realised. Ultimately, by combining colony measurements, mite measurements and climate factors, it should be possible to predict for each region, when the climate effects result in more mite production than under normal (average) years.

Pesticides and pests / invasive pests

The influence on bee health from agrochemicals and other use of chemicals with potentially bad effects on colony health is still not well understood. Clearly, there may be synergistic effects between substances we know very little about, and sublethal effects from chemicals may still impact on colony productivity and winter survival. In particular, it needs to be studied if the impact of certain pathogens on honeybee

colony vitality is influenced by a chemical pressure from outside that alone cannot explain colony mortality.

The new invasive species, *Vespa velutina*, which recently invaded France, represents a new threat to honeybees in regions, where it is expected to expand. This species, like many other Asian hornets, is a fierce predator of honeybees. Colony health, vitality, and ability to respond to various levels of predation pressure needs to be investigated since damage may be more or less severe in relation to these factors.

To be able to produce reproducible and comparable data on all aspects considered it is essential that common protocols are used. Antonio Nanetti and Ingemar Fries will suggest a protocol with requirements for studies of the effects from interactions between pathogens in honey be colonies. A draft version will later be sent out for comments within WG2.

WG3: Environment & Beekeeping

Participants

Bouga Maria, Christina Emmanouil, Crailsheim Karl, Gregorc Ales, J. van der Steen, T. Blacquière, Piotr Medrzycki

Karl Crailsheim welcomed the participants of the WG3 meeting and gave a short introduction on the proposed activity of the group. 23 researchers have expressed interest to participate to the WG3 and few of them have prepared proposal to the COST project and presentations to the meeting. He exposed following questions: Who is present and willing to contribute to the collaboration with support of the project and their research regardless the COST project. The research themes conducted in Graz were presented: American foulbrood, larval and thermal behaviour and proposed grants on CCD Survey in Austria, electromagnetic fields and honeybee nutritional requirements.

Desired cooperations were expressed from:

- James Ellis, Jeff Pettis (nutritional requirements, CCD colonies in the US, sublethal effects),
- Rudolf Moosbeckhofer: CCD in Austria,
- Ales Gregorc (overwintering and nutrition)
- M.P. Chauzat (toxification)
- Maria Bouga (DNA-integrity)

During the COST project application following specific research focuses were established:

- Nutrition (larval, adult)
- Beekeeping
- Intoxication
- Overwintering & Colony Development
- Viability & Disease Susceptibility (in coordination with WG 2)

Studies are proposed to be conducted in different aspects as described in application for the COST project by different members of the WG3.

There are overlaps with WG 1 (Monitoring & Diagnosis) in specific areas (establishing of a questionnaire, survey modalities, significance of environmental factors - supplemental feeding…). To explore already existing experiences in participating research groups.

Colony losses and present research activities at Agricultural Inst. Of Slovenia were presented by Ales Gregorc. Approximately 30 % colony losses were monitored till now; and colony mortality is also economic problem. Research activities of sub-lethal effects on bee were presented using toxicological and cell biology methods. Studies were preformed on colony and bee level, detecting cell death in situ in tissue and heat shock proteins localization. Collaboration in exploring expertise would be an advantage for the WG.

Questionnaire on Colony losses 2006 – 2007 and planned research 2008 was presented by J. van der Steen. About 10 % colony losses were detected during questionnaire. Different diseases, control and technological aspects were considered. In proposed research different Varroa treatment strategies will be explored (year round treatment, post summer, winter treatment and conditions of the bees will be determined by vitellogenin titre in haemolymoph).

Tjeerd Blacquière and Sjef van der Steen presented Coloss: Bee diseases survey monitored: Varroa, AFB, EFB, Nosema (*apis & ceranae*), viruses: KBV, DWV, BQCV, SBV, CPV, APV, IV, Chalk brood, Stone brood, *Acarapis woodi*, heavy metals, honey samples(pollen spectrum). Future plans are in establishing relations between *Varroa* infestation rate and other pathogens (viruses, AFB, ...).

Expertise in the role of agrochemicals in bee losses was presented by Piotr Medrzycki. Effects of agrochemicals and GMOs to bees were studied in field, semifield and lab trials on toxicity and sublethal effects (behaviour etc.); Lab honeybee brood rearing and use of honeybees as bioindicators of environmental contamination (agricultural, urban, industrial, military etc.) were presented. Future research will be directed in study the synergisms of different potential bee loss factors, new method for testing hazard of agrochemicals to forager bees, detection of explosive materials and establishing large-scale monitoring system for collecting data about bee losses in Italy.

Genotoxicity as a marker of general health of the honeybee was presented by Christina Emmanouil and Maria Bouga. Biomarkers (DNA damage) of effect measure a toxic response or disease progressions as a consequence of exposure to noxious substances/organisms were used. Single cell gel electrophoresis (Comet assay) was used and further development of the technique will be applied for insect cells, various bee tissues and could be a tool as a marker of non-specific genotoxicity.

Researchers who were not at the meeting presented their research plan for the WG 3:
Jurek Wilde: work on the development and productivity of bee colonies of *Apis mellifera carnica* and pure *Apis mellifera mellifera* (spared ganes of Polish line), observation of the development of *V. destructor* and *N. apis,* experience in breeding with the use of insemination and in the selection of bees resistant to *V. destructor,* testing the resistance of Varroa mites to acaricides.

Meral Kence: discrimination of the honeybee colonies belonging to different subspecies, ecotype and understanding of reasons of colony losses by using genetic markers such as microsatellites, SNP analyses, monitoring environmental factors (pollination, GM plants, pollution (pesticides), climate change (drought affecting flora), pathogens, beekeeping practices. Genetic determination, mapping, selection, behavioural defence mechanisms against parasites, assessment of differences in overwintering adaptations and related energy metabolism.

Robert Paxton: novel methods of honey bee disease identification, impacts of honey bee diseases on individual and colony fitness, pollination from the perspective of ecosystem services.

Franco Mutinelli: optimization of analytical methods to detect active principles possibly responsible for honeybee losses, environment (beekeeping): evaluation of apicultural techniques.

Chauzat Marie-Pierre: European knowledge on pesticide uses and getting reliable data to better know the potential intoxication risks, to understand how bees react to environmental stress, cellular markers will be studied in different conditions: bees exposed to pesticides, pathogens or nutritive stresses.

Ken Tan: a monitoring net through out China, study the pathogens in local *Apis mellifera* colonies, carry out a series study on the environment and honeybee, also do some bee breeding programs.

Jeff Pettis: research is underway in the U.S. to determine the factors involved in colony loss, nutrition, pesticide exposure, migratory stress and pathogen load, experimental manipulation are planned in commercial beekeeping operations with an overall goal of improving colony survival.

Yves Le Conte: studying the effects of Varroa mites on honeybee gene expression, the effects of other pathogens and stress (pesticides and beekeeping) and their interactions. The pattern of gene expression can be used as diagnostic but also in honeybee selection programs; to set up a molecular diagnostic tool including dosages of the different pathogens and gene markers of stress.

Per Kryger: diagnosis of pathogens in honey bees, sampling of bees, monitoring of colony survival will be carried out by the beekeeping associations, bee vitality is an aspect we will promote.

Ralph Büchler: developing standard test protocol for field tests on vitality, field testing for differences and genotype-environment (diseases, nutrition, management systems…) interactions in the vitality of different European bee strains, comparison of different mating systems for its effects on bee vitality, colony management systems to improve the vitality and survival rate of colonies.

James Ellis: determining (1) nutrition effects on honey bee disease susceptibility, (2) sublethal effects of chemicals on honey bee disease susceptibility, and (3) IPM control of small hive beetles and other diseases/pests.

Diana Sammataro: the aspect of nutrition and honey bee health, studying high fructose corn syrup (commonly fed by commercial operations) and it's components to determine if this is detrimental to bees, sampling pollen from major crops pollinated by bees in the U.S. and assessing protein and amino acid content and how it relates to overall bee health.

Final discussion and conclusions
All participants were happy about the meeting, and discussions concerning ongoing and planned projects. Members support ongoing cooperation. Several research groups are currently performing and adopting the same methods which could be applied for different research aims. The group agreed that common goals could be defined and it could work together to reach them in a coordinated way.

Protocol: Aleš Gregorc

WG 4: Diversity and Vitality

Discussion summary
We state that there is huge honeybee diversity at subspecific, biological, behavioural, morphological, molecular levels in Europe and other areas of the world where *Apis mellifera* is native. We identify the urgent need for conservation and 'propagation' of this diversity, and to this aim additional research on honey bee biodiversity is necessary.

The reduction of biodiversity may reduce vitality. Furthermore, current breeding schemes have given scarce consideration to bee vitality. The effects of traditional selection strategies on vitality are unknown and may be ambiguous. According to the breeding traditions present in different countries, effects on honey bee diversity and vitality may be differing.

We therefore identify the need for a re-evaluation of current breeding and selection schemes, which need to be adapted to an 'integrated approach' that should include bee vitality parameters.

To make this feasible on a large scale we need to develop a relatively simple 'field test kit' for breeding purposes.

The question is: how can vitality of a honey bee colony be tested for? Considering the practical difficulty of testing for adaptation to climatic change and genetic susceptibility to pesticides, we suggest focusing on how bees cope with pathogens.

Among these, we identify *Varroa destructor* as the major factor that currently reduces bee vitality worldwide, and for this reason we suggest to first focus on Varroa tolerance. As a starting point we plan to use the methods developed by the German Tolerance Breeding group, such as monitoring the build-up of mite populations and the assessment of brood hygiene. However these methods need to be both adapted to different regions, different subspecies and different beekeeping management strategies, and standardized so as to be comparable and effective.

Methods to assess tolerance towards other pathogens could be developed in a later stage.

We identify the importance of developing regional damage thresholds necessary to make treatment decisions and reduce the input of chemicals in breeding operations.

We identify the need for a change in beekeeping management, in terms of aiming towards concerted pest management at local level. This can be achieved by the dissemination of new technology and management knowledge into the beekeeping community, which must be circulated within the community itself. We therefore ought to encourage the beekeepers to a 'concerted action' to improve the communication within the beekeeping community.

Finally, biodiversity and vitality provide the background for all other working groups within COLOSS. All ideas and findings must be proven in the ‘real world’.

Concluding remarks from the joint discussion with WG 3 – environment and beekeeping

The two working group share common interests, research fields and goals, such as

- Improvement of the overwintering ability of colonies
- Improvement of bee vitality
- Development and use of diagnosis tools
- Bee nutrition
- Resistance selection
- Sharing of molecular tools

During the joint discussion, it became apparent that projects with a similar background were already going on in different labs and ideas for cooperation emerged between those groups. We envision the sharing of ideas, materials and tools among these projects.