4th European Conference of Apidology (EURBEE 2010)

September 07-09, 2010
Middle East Technical University
Cultural & Convention Center

ANKARA, TURKEY

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European Association for Bee Research

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preface

Proceedings of the fourth European Conference of Apidology, EurBee2010 contain the texts or abstracts of presentations of participated researchers from Europe as well as many other countries. All share the interest in different aspects of mysterious lives of bees brings them together to share their recent research results and exchange ideas. Remembering Norberto Milani and the statement he made in the Introduction of First EurBee Conference in Udine in 2004, "ambition of the conference was to bring together all the European researchers in different fields of Apidology and Apiculture, offering an opportunity to present recent advances achieved in Europe and promoting a multidisciplinary approach to open problems" six years later we realize that the problems grew as well as achievements. The problems of pervasion of diseases due to bee's borderless life and unknown factors causing unusual massive deaths of the bees concern not only the scientists but everyone.

The Fourth EurBee Conference is meeting in Ankara, in the middle of Anatolia where the most ancient civilizations culminated and the ancient beekeeping in Hittite Old Kingdom (1650-1500BC) where a part of written law-text reads: "if anyone steals bees in a swarm, pay shekels of silver, anyone steals bee hive would have been exposed to bee-sting, later he shall pay 6 shekels of silver." This shows the economical value of bees that lead to crime and legislation at a very early time.

The fourth EurBee Conference has attracted more than 250 participants from 40 countries, with 140 oral and 100 poster presentations under 15 symposia . One of the unique features of the conference is the organization of three workshops; 2 sessions on "Behavioral Plasticity" and "BeeDoc" which are supported by NSF and EU respectively.

We thank to eight plenary speakers and all participants of the EurBee2010 conference for their valuable contributions. Thanks are also due to incentive approach of the Rector and other administrative staff of the Middle East Technical University, for logistic and moral support. We thank to our sponsors not only for their material support but sharing the excitement.

Hope this conference be succesful for participating scientists and benefical for all humanity.

September 2010

Meral Kence

Chair of the Local Organizing Comittee

PROGRAM AT A GLANCE

6 September Monday		**7 September Tuesday**	
	Morning	Opening Session	
		Plenary Presentation N Koeniger	
Arrival		*Themes*	*Symposia*
		Losses:	1. Varroa and viruses
		Conservation:	5. Diversity of bees
		Biol. Ecol.:	7. Bees and pollination
Registration	Lunch		
13:00-19:00	Afternoon	Plenary Presentation K Delaplane	
		Themes	*Symposia*
		Losses:	2. Nosema and viruses
		Beekeeping:	13. Bees in Turkey
		Biol. Ecol.:	9. Devo. behav. plasticity
		Welcome Address	
	Evening	Plenary Presentation Z Huang	
		Reception	

	8 September Wednesday	
Morning	Plenary Presentation P Neumann	
	Themes	*Symposia*
	Losses:	3. Monitoring, diagnostics
	Beekeeping:	12. Bee products
	Biol. Ecol.:	11. Nutrition and physiology
Lunch		
Afternoon	Plenary Presentation R Moritz	
	Themes	*Symposia*
	Conservation:	6. Drivers of bee loss
	Open:	15a: Bee reproduction
	Biol. Ecol.:	10. Learning & memory
Evening	Plenary Presentation A Hefetz	
	Workshop: Behavioral plasticity	

PROGRAM AT A GLANCE

	9 September Thursday		**10 September Friday**
Morning	Plenary Presentation B Smith		Daily Excursions
	Themes	*Symposia*	
	Losses:	4. Side effects of pesticides	
	Open:	15b. Contributions	
	Open:	14. BeeDoc	
	Biol. Ecol.:	8. Genome & genomics	
Lunch			
Afternoon	Plenary Presentation M Doğaroğlu		
	Closing Session		
	Workshop: Behavioral plasticity		
	Workshop: BeeDoc		

Plenary Presentations

Nikolaus Koeniger* and Gudrun Koeniger
Consensus tree and controversial polarity; old and new approaches to honey bee evolution

Keith S. Delaplane
Addressing bee decline through the Managed Pollinator CAP - a nationally-coordinated research and education program

Plenary Presentation
Zachary Y. Huang, Ting Zhou, Xianbing Xie, Shuangxiu Huang, Denis Anderson, Yves Le Conte, Zhijiang Zeng
Varroa mite reproductive biology

TUESDAY 09:50-12:50 HALL A

Theme I. BEE LOSSES: Symposium 1. Pests, Pathogens and bee loss I: focusing on Varroa and viruses

Organizers: Ilan Sela and Peter Neumann

09:50 1.1. Varroa destructor mite reproduction in honey bee colonies surviving mite infestation in Sweden and France
Barbara Locke*, Yves Le Conte, Ingemar Fries

10:10 1.2. The deformed Wing Virus (DWV) is poorly pathogenic for queen honey bees (Apis mellifera L.)
Laurent Gauthier*, LaurentMarc Ravallec, Magali Tournaire, Benjamin Dainat, François Coisserans, Max Bergoin and Joachim de Miranda

10:30 1.3. Involvement of deformed Wing Virus (DWV) and Varroa destructor virus (VaDV) in the deformed wing syndrome of the honey bee
Nor Chejanovsky*, Victoria Soroker, Naama Tzioni

10:50 - 11:10 Coffee Break

11:10 1.4. Impact of virus infections on vitellogenin expression: mechanism contributing to colony losses?
Benjamin Dainat*, Laurent Gauthier, Jay D. Evans, Peter Neumann

11:30 1.5. Remebee - RNAi based product-line targeting major culprits in Beekeeping
Eyal Ben-Chanoch, Eitan Glick, Nitzan Paldi and Gal Yarden*

11:50 1.6. Application of Porous Ceramics in Delivering Lemongrass Oil to Control Mites and Bee Pathogens
Bongkot Booppha,Sukum Eittsayea,Kamolpan Pengpat and Panuwan Chantawannakul

12:10 1.7. Negative side effects of viruses and Varroa destructor on honey bee colony survival in Belgium
Bach Kim Nyguyen*, Magali Ribière, Dennis vanEngelsdorp, Claude Saegerman, Yves Brostaux, Jean-Paul Faucon, Eric Haubruge

12:30 1.8. Relationships between integrated viral sequences and bee behaviour and horizontal transfer of RNAi in bees
Ilan Sela, Eyal Maori, Yael Gabrian, Sharon Shafir*

TUESDAY 09:50-12:50 HALL B

Theme II. DIVERSITY AND CONSERVATION: Symposium 5. Diversity of bees
Organizers: Per Kryger and Lionel Garnery

09:50 5.1. Revealing molecular biodiversity within East-European honey bees
Pilar De La Rúa*, Irene Muñoz

10:10 5.2. Barcoding: It is a useful tool for the discrimination of honey bee, A. mellifera populations?
Maria Bouga*, Per Kryger

10:30 5.3. A Europe-wide experimental approach to reveal genotype-environment interactions
Marina Meixner*, Ralph Büchler

10:50 - 11:10 Coffee Break

11:10 5.4. New data about allozyme variability of Bulgarian honey bees
Evgenia Ivanova*

11:30 5.5. Intracolony selection of the Dark European honey bee for disease resistance
Norman L. Carreck*, L. Alton, Francis W. L. Ratnieks

11:50 5.6. Biodiversity of the honeybee population from La Palma (Canary Islands, Spain) and influence of the conservation program on a natural mating area
Irene Muñoz*, Pilar de la Rúa

12:10 5.7. Genetic Diversity of the honeybee populations (Apis mellifera L.) from Turkey and Iran based on 20 microsatellites
Fulya Özdil*, Cengiz Erkan, Kadir Sarıyüz, Mehmet Ali Yıldız

12:30 5.8. Functionally different sex-alleles in honeybee populations: a tool to determine allele frequencies
H. Michael G. Lattorff*, Marcel Medrano, Marie-Jose Duchateau, Robin F.A. Moritz

TUESDAY 09:50-12:50 HALL D

Theme III. BEE BIOLOGY and ECOLOGY Symposium 7. Bees and pollination
Organizers: Pierre Rasmont and Murat Aytekin

09:50 7.1. The role of bees as pollinators for Vicia faba in the UK
Claire Hutchins-Lee*, Jacobus Biesmeijer, William Kunin

10:10 7.2. A Biogeographical Analysis of the Genus Evylaeus Robertson (Hymenoptera: Halictidae) of Turkey"
Fatih Dikmen*, Ahmet Murat Aytekin

10:30 7.3. Host-plant specialization
Denis Michez

10:50 - 11:10 Coffee Break

11:10 7.4. Effects of hedge connectivity and adjacent crop fields on the pollination of shrub species
Aniko Kovács*, Sebastian Haenke, Peter Batáry, Andrea Holzschuh, Birgit Meyer, András Báldi, Teja Tscharntke

11:30 7.5. Bumblebee diversity and flower visits on experimental long-term set-asides in southern Finland
Eeva-Liisa Alanen*, Terho Hyvönen

11:50 7.6. Temporal dynamics of the male effective population size in bumblebees (Hymenoptera:Apidae)
Stephan Wolf*, Theresa Toev, Ruby L. V. Moritz, Robin F. A. Moritz

12:10 7.7. Genetic colony structure and male production in the neotropical bumblebee Bombus wilmattae (Hymenoptera: Apidae)
Anett Huth-Schwarz*, F. Bernhard Kraus, Robin F.A. Moritz

12:50 - 14:00 Lunch Break

TUESDAY 14:00-14:50 CONFERENCE HALL

Plenary Presentation
Keith S. Delaplane
Addressing bee decline through the Managed Pollinator CAP - a nationally-coordinated research and education program

TUESDAY 14:50-18:10 HALL A

Theme I. BEE LOSSES: Symposium 2. Pests, Pathogens and bee loss II: focusing on Nosema and viruses
Organizers: Mariano Highes, Raquel Martín-Hernández and Magali Ribière

14:50 2.1. Young and vulnerable - chalkbrood infection experiments in honey bee larvae of variable age
Annette Bruun Jensenn*, Jørgen Ellenberg

15:10 2.2.Interaction between Nosema spp. and a neonicotinoid affect honey bees at the individual and social level
Claudia Dussaubat*, Cedric Alaux, Alban Maisonnasse, Jean-Luc Brunet, Sylvie Tchamitchan, Fanny Mondet, Marianne Cousin, Julien Brillard, Luc Belzunces, Erica Plettner, and Yves Le Conte

15:30 2.3. Beepath, a newborn association of apidology
Gianluigi Bressan*, Antonio Felicioli, Antonio Nanetti, Besana A, Barone S, Capua C.

15:50 2.4. Prevalence of pathogens and tracheal mite in honey bees in Japan
Toki Taku, Yuriko Kojima, Tomomi Morimoto, Mikio Yoshiyama, Kiyoshi Kimura, and Tatsuhiko Kadowaki*

16:10 - 16:30 Coffee Break

16:30 2.5. What has Nosema got to do with losses? Monitoring both Nosema species in the UK
Giles Budge*, Michelle Powell, Kat Roberts, Ian Adams, Ben Jones, Gay Marris, Lynn Laurenson, Selwyn Wilkins, Stéphane Pietravalle, Mike Brown

16:50 2.6. Effect of a diet, Nosema and virus infections on honeybee (Apis mellifera carnica) colonies
Maja Ivana Smodis Skerl*, Mitja Nakrst, Ales Gregorc

17:10 2.7. Negative effects of Nosema infection in honey production and vitality of honey bee colonies (Apis mellifera iberiensis) in Spain
Cristina Botías*, Raquel Martín-Hernández, Aránzazu Meana, Mariano Higes

17:30 2.8. Nosema ceranae as the agent of Type C Nosemosis in Apis mellifera honeybees
Raquel Martin- Hernández*, Aránzazu Meana, Mariano Higes

17:50 2.9. Effects of queen supersedure in Nosema spp. infected honey bees (Apis mellifera iberiensis) colonies
Cristina Botías*, Raquel Martin-Hernández, Aránzazu Meana, Mariano Higes

TUESDAY 14:50-18:10 HALL B

Theme IV. BEEKEEPING AND BEE RESEARCH: Symposium 13. Beekeeping and bee research in Turkey
Organizers: Devrim Oskay and Ethem Akyol

14:50 13.1. Industrial apiculture in the Jordan valley during Biblical times with Anatolian honey bees
Guy Bloch*, Tiago M Francoy, Ido Wachtel, Nava Panitz-Cohen, Stefan Fuchs, Amihai Mazar

15:10 13.2. A study on determining the effect of drug treatment season on Varroa (Varroa destructor) population growth
Ethem Akyol, Halil Yeninar*, D. Ali Ceylan, Nuray Şahinler

15:30 13.3. Wing shape analysis of honey bee subspecies distributed in the Middle East using landmark method
Ayça Özkan*, Mohammad G. Moradi, İrfan Kandemir

15:50 13.4. How changing population dynamics affect honey bee colony performance
Devrim Oskay*, Sam Hapke, Muhsin Doğaroğlu, Walter S. Sheppard

16:10 - 16:30 Coffee Break

16:30 13.5. Genetic diversity of honey bee ecotypes in Turkey based on microsatellites
Meral Kekeçoğlu*

16:50 13.6. Morphological characteristics of the honey bee (Apis mellifera L.) samples from the border region in Turkey with Bulgaria
Peter Nentchev*, Ibrahim Çakmak, I. Jeliazkova, S. Seven Cakmak, L.Jordanova

17:10 13.7. Research of honey bee colony losses and deaths in Marmara region
Hasan Hüseyin Ünal*

17:30 13.8. Nosema cerenae and Nosema apis in colony collapsed apiaries of Hatay
Mustafa N. Muz*, Dilek Muz

17:50 13.9. Migratory Beekeeping in Turkey and Related Problems
Aslı Elif Sunay*, Taylan Samancı

TUESDAY 14:50-18:10 HALL D

Theme III. BEE BIOLOGY and ECOLOGY: Symposium 9. Developmental and behavioral plasticity in eusocial bees
Organizers: Osman Kaftanoglu and Florian Wolschin

14:50 9.1. Behavioural plasticity underlies the solitary-eusocial transition in the socially polymorphic sweat bee Halictus rubicundus
Robert Paxton*, Antonella Soro, Cathy Bridge, Jeremy Field

15:10 9.2. Functional genomic approaches to unravel the physiological basis of honey bee caste development
Klaus Hartfelder*

15:30 9.3. Flexibility of division of labor in honey bees: all explained by a simple model
Zachary Y. Huang*

15:50 9.4. Proteotyping division of labor in worker honeybees
Florian Wolschin*

16:10 - 16:30 Coffee Break

16:30 9.5. Effects of carbohydrates on the development and sugar responsiveness of honey bees (Apis mellifera L.) reared in vitro
Osman Kaftanoğlu, Julie A Mustard, Ethem Akyol, Tim A. Linksvayer, Robert E. Page Jr.

16:50 9.6. The putative role of male sex pheromones in bumblebee cuckoo-host interactions
Patrick Lhomme*

17:10 9.7. Larval and nurse worker control of developmental plasticity in honey bee queens and workers
Timothy Linksvayer*, Osman Kaftanoğlu, Ethem Akyol, Gro V. Amdam, Robert E. Page

17:30 9.8. Socially-mediated plasticity in circadian rhythms and the molecular clockwork
Guy Bloch*

17:50 9.9. Functional mapping of the circadian network and its role on the sleep-like state
Jose Luis Agosto

TUESDAY 18:20-19:10 CONFERENCE HALL

Plenary Presentation
Zachary Y. Huang, Ting Zhou, Xianbing Xie, Shuangxiu Huang, Denis Anderson, Yves Le Conte, Zhijiang Zeng
Varroa mite reproductive biology

TUESDAY 19:15-19:30 CONFERENCE HALL

Welcome Address

TUESDAY 19:30-21:30 DINING HALL

Welcome Reception

WEDNESDAY 08:30-09:20 CONFERENCE HALL

Plenary Presentation
Peter Neumann
Honey bee colony losses and the decline of beekeeping

WEDNESDAY 09:20- 13:20 HALL A

Theme I. BEE LOSSES: Symposium 3. Monitoring, Diagnostics, and Pathogens
Organizer: Romée van der Zee

09:20 3.1. Bee Mortality and Bee Surveillance in Europe
Marie Pierre Chauzat*, Pascal Hendrikx, Marion Debin, Peter Neumann, Ingemar Fries, Wolfgang Ritter, Mike Brown, Franko Mutinelli, Yves Le Conte, Ales Gregorc

09:40 3.2. Honeybee Colony Losses 2009-10 in 23 countries using mainly the COLOSS 2009-10 questionnaire
Romée van der Zee*

10:00 3.3. Detection of the major honeybee pathogens by Multiplex PCR: from honeybees to other organisms
Jérôme Carletto*, Philippe Blanchard, Frank Schurr, Patrick Drajnudel, Marie Pierre Chauzat, Jean-Paul Faucon, Magali Ribière

10:20 - 10:40 Coffee Break

10:40 3.4. Detection of pathogens in honeybee mortalities observed in France during 2007 to 2009
Magali Ribière*, Jérôme Carletto, Philippe Blanchard, Frank Schurr, Olivier Celle, Aurore Chevin, Jean-Paul Faucon

11:00 3.5. Can we think differently talking about European foulbrood causalities?
Violeta Santrac*, Tomljanovic Zlatko

11:20 3.6. Why do bees survive well in Northern countries in spite of unkind environment?
Lauri Ruottinen*, Lassi Kauko

11:40 3.7. Estimation of honeybee colony losses within professionnal beekeepers in France during winter 2008/2009
Fabrice Allier*, C. Holzmann, V. Britten, P. Jourdan, Julien Vallon

12:00 3.8. First detection of honey bee pathogens in nests of the oriental hornet (Vespa velutina collected in France)
Marie Pierre Chauzat

12:20 3.9. Prevalence of viruses and Nosema spores in reared queens, Apis ellifera carnica (Pollmann, 1879
Maja Ivana Smodis Skerl *, Vesna Lokar, Mitja Nakrst, Ales Gregorc

WEDNESDAY 09:20- 13:00 HALL B

Theme IV. BEEKEEPING AND BEE RESEARCH: Symposium 12. Bee Products

Organizer: Andreas Thrasivoulou

09:20 12.1. The estimation of unifloral Acacia honeys harvested in Poland on the basis of their pollen analysis and fructose/glucose ratio
Dariusz Teper*, Helena Rybak -Chmielewska, Teresa Szczesna, Ewa Was

09:40 12.2. Determination of origin in honey by aroma profile analysis
Aslı Elif Sunay*, Ilnur Atalay Coşkun

10:00 12.3. Flavours of heather (Ericaceae) honeys: organoleptic and instrumental analyses
Ana Pascual-Mate*, M.A. Fernandez-Muino, A.V. Gonzalez-Porto, V. Leon-Ruiz, T. Marin-arroyo, and M. Teresa Sancho

10:20 - 10:40 Coffee Break

10:40 12.4. Non-aromatic organic acids of honey: significance and analysis
I. Mato, J.F. Huidobro, S. Suarez-Luque, J. Simal-Lozano, M.A.Fernandez-Muino, and M. Teresa Sancho*

11:00 12.5. Efforts to explain the great variability of diastase activity in monofloral honeys
Andreas Thrasyvoulou*, Crysoul Tananaki, Goras Georgios

11:20 12.6. Migration of residues of chloramphenicol from contaminated beeswax foundations to honey
Reybroeck Wim Frans Jacobs*, Els Daeseleire

11:40 12.7. The specific chemical profile of Maltese propolis and its antimicrobial activity
Vassya Bankova*, Milena Popovaa, Boryana Trushevaa, Daniela Antonovaa, Simone Cutajarb, David Mifsudc, Claude Farrugiab, Iva Tsvetkovad, Hristo Najdenskid

12:00 12.8. Bee products in human medicine - the need for standardization
Norman L. Carreck*, Sarah L. Jones, Rose A. Cooper.

12:20 12.9. Characterization of a propolis extract from a French landscape mosaic marshlands/grove/forest
Benjamin Poirot*, V. Léonard-Neversa, H. Hachetb, G.Grellierb.

WEDNESDAY 09:20- 13:00 HALL D

Theme III. BEE BIOLOGY and ECOLOGY Symposium 11. Nutrition and physiology in bees
Organizers: Karl Crailsheim and Yves Le Conte

09:20 11.1. Is there 'revers' food flow from brood cells to adults in honey bees?
Robert Brodschneider*, Robert Jutta Vollmann, Ulrike Riessberger-Gallé, Karl Crailsheim

09:40 11.2. The impact of nutritional protein on the honey bee - a review
Karl Crailsheim*

10:00 11.3. Nutrigenomics in honey bees
Cedric Alaux*, Christelle Dantec, Hughes Parrinello, Yves Le Conte

10:20 - 10:40 Coffee Break

11:00 11.4 Strategies of energetic and thermal optimization of foraging honeybees
Anton Stabenheiner*, Helmut Kovac

11:20 11.5. Impact of transgenic proteins, Bt-pollen and colony affiliation on survival and longevity of honey bee workers
Stephan Härtel*, Alexandra Kästner, Ingolf Steffan-Dewenter

11:40 11.6. Does in vitro larval rearing conditions influence adults behavior?
Pierrick Aupinel *, C. Bordier, D. Fortini, J. F. Odoux, A. Decourtye

12:20 11.7. Comparison of two methods to assess effects of insecticides on hypopharyngeal gland development of honey bee
Pierrick Aupinel *, D. Fortini

13:00 - 13:45 Behavioral Plasticity Workshop: Session 1 Hall D

12:40 - 13:45 Lunch Break

WEDNESDAY 14:00- 14:50 CONFERENCE HALL

Plenary Presentation
Robin F. A. Moritz
Reproductive conflicts in honey bees: how to kill a queen and get away with it

WEDNESDAY 14:50- 17:50 HALL A

Theme II. DIVERSITY AND CONSERVATION: Symposium 6. Drivers of bee loss in Europe and impacts for society.
Organizers: Simon G. Potts and Koos Biesmeijer

14:50 6.1. The effect of climatic variation on the mountain bumblebee fauna
Stephanie Iserbyt*, Pierre Rasmont

15:10 6.2. Are droughts and heatwaves leading to local extinctions of bumblebees?
Pierre Rasmont*, Stephanie Iserbyt

15:30 6.3. Resource use of maize pollen by honey bees in differentially structured landscapes
Stephan Härtel*, Stephan Nadja Danner, Ingolf Steffan-Dewenter

15:50 - 16:10 Coffee Break

16:10 6.4. Landscape complexity and flowering herbs enhance wild bee density and sweet-cherry yield
Andrea Holzschuh*, Jan-Hendrik Dudenhöffer, Teja Tscharntke

16:30 6.5. Does bee decline matter? A framework for characterizing a pollinator's agricultural importance
Keith S. Delaplane*

16:50 6.6. Apis florea -the future dominant honey bee in Europe?
Sharoni Shafir*, B. Roy Kaspi, B. Aliza Fleischer, Yael Mandelik

17:10 6.7. Bees in intensive cereal farming systems: landscape composition influences colony dynamics
Jean- François Odoux*, Mickael Henry, G. Caro, Thierry Tamic, Clovis Toullet, E. Peyra, D. Derelle, Pierrick Aupinel, Vincent Bretagnolle

17:30 6.8. Novel management to boost bee habitat quality in existing grass buffer strips
Robin Blake*, Duncan B. Westbury, Ben A. Woodcock, Peter Sutton, Simon, G. Potts

WEDNESDAY 14:50- 18:10 HALL B

Theme III. BEE BIOLOGY and ECOLOGY : Symposium 10. Learning and memory in honey bees
Organizers: Charles Abramson and Tuğrul Giray

14:50 10.1. Mechanisms of learning and memory in honey bees
Tuğrul Giray*

15:10 10.2. Optic flow informs distance but not profitability for honey bees
Sharoni Shafir*, Andrew B. Barron

15:30 10.3. Some issues in the cognitive interpretation of invertebrate behavior
Charles Abramson*

15:50 - 16:10 Coffee Break

16:10 10.4. Modification of olfactory learning and memory induced by siRNA targeting nicotinic acetylcholine subunits in the honeybee
LOUIS Thierry1, MUSSO Pierre-Yves1, OLIVEIRA Sabrina1, GARREAU Lucile1, AHIER Arnaud2, GIURFA Martin, DISSOUS Colette, RAYMOND-DELPECH Valérie1 & GAUTHIER Monique1

16:30 10.5. Foraging prefence and flower handling in honey bee subspecies
Ibrahim Cakmak, Tuğrul Giray*, Charles I. Abramson, Harrington Wells

16:50 10.6. Memory dynamics of honey bees
Martin Giurfa*

17:10 10.7. Operant conditioning protocols in honey bees
Michel Sokolowski* Charles Abramson

17:30 10.8. Olfactory learning in honey bees
Brian H. Smith*

WEDNESDAY 14:50- 18:10 HALL D

Theme V. OPEN SESSIONS: Symposium 15a. Open session
Organizer: Meral Kence

14:50 15a.1. Preliminary results from a study on Balkan honey bees' genetic variability using isoenzymic approach
Evgenia Ivanova*, Maria Bouga, Plamen Petrov, Mica Mladenovic, Sladjan Rasic, Leonidas Charistos, Fani Hatjina

15:10 15a.2. Breeding and conservation projects in Italy: economy and science co-operate to save biodiversity
Cecilia Costa, Raffaele Dall'Olio*, Marco Lodesani

15:30 15a.3. Analysis of the relation between pathogens and the loss of bee colonies (results 2008-2009)
Krystyna Pohorecka, Andrzej Bober, Dagmara Zdanska*, Marta Skubida

15:50 - 16:10 Coffee Break

16:10 15a.4. Territorial biodiversity and consequences on physico-chemical characteristics of collected pollen in bee colonies
Jean-François Odoux*, D. Feuillet, D. P. Aupinel, Y. Loublier, J.N. Tasei, C. Mateescu

16:30 15a.5. Longitudinal comparative study of colonies headed by US and Australian queens in US East coast operations
Dennis vanEngelsdorp*, Eugene J. Lengerich, and Jeff Pettis

16:50 15a.6. Origin of queens as a cause of colony losses
Maja Drazic*, Janja Filipi, Lidija Svecnjak, Dragan Bubalo, Nikola Kezic

17:10 15a.7. Nectar flow of some sunflower hybrids
Marcel Policka*, Alla Faková, Róbert Chlebo

17:30 15a.8. What is the relation between Israeli Acute Paralysis Virus and Colony Collapse Disorder? Utility of IAPV detection
Deborah Kukielka Zunzunegui*, Marina Vicente Rubiano, José Manuel Sánchez-Vizcaíno

WEDNESDAY 18:00- 18:50 CONFERENCE HALL

Plenary Presentation
Abraham Hefetz
Pheromone involvement in reproductive competition in the bumblebee Bombus terrestris

WEDNESDAY 19:00- 19:45 HALL D

Behavioral Plasticity Workshop: Session 2

THURSDAY 08:30- 09:20 CONFERENCE HALL

Plenary Presentation
Brian H. Smith
Mechanisms of learning and memory for complex floral odors

THURSDAY 09:20-12:40 HALL A

Theme I. BEE LOSSES: Symposium 4. Side effects of pesticides to bees
Organizers: Monique Gauthier and Claude Collet

09:20 4.1. A new method of assessing pesticides and repellents in honey bees
Charles Abramson*

09:40 4.2. Imidacloprid enhances outbreak of nosemosis in honey bees
Jasna Krajl*

10:00 4.3. Do varroa mites and pesticides synergize to affect bee larvae?
Ales Gregorc*, Jay D. Evans, James Ellis

10:20 - 10:40 Coffee Break

10:40 4.4. Neonicotinoid toxicity towards bees
Daniela Laurino*, Aulo Manino, Augusto Patetta, M. Ansaldi, Marco Porporato

11:00 4.5. Type I and type II pyrethroids modify honeybee antennal neurons function
Aklesso Kadala*, Mercedes Charreton, Ingrid Jakob, Yves Le Conte, Claude Collet

11:20 4.6. Assessment and management of pesticide risks to bees- an ongoing scientific and legal challenge
Pieter A. Oomen*

11:40 4.7. Is it possible to use Apis mellifera in order to certify environmental, agricultural and food quality?"
José Antonio Ruiz*, J. M. Fernández Perejon, M. Gutiérrez, C. Porrini, A. G. Sabatini, F. Puerta

12:00 4.8. Effect of transgenic maize pollen on survival and weight of in vitro reared honey bees
Harmen P. Hendriksma*, Stephan Härtel, Ingolf Steffan-Dewenter

12:20 4.9. Honey bees: recording individual behaviour using microchips
Axel Decourtye, C. Bagnis, James Devillers, F. Brun, Pierrick Aupinel, Julie Fourrier, M. Gauthier

THURSDAY 09:20-12:20 HALL B

Theme III. BEE BIOLOGY and ECOLOGY : Symposium 8. Bee genome and Genomics
Organizers: Michael Lattorff and Cédric Alaux

09:20 8.1. Insights into the molecular evolution of sex determining genes in corbiculate bees
Martin Hasselmann*

09:40 8.2. Evidence for a genetic basis of shift work in honey bee pollen foragers (Apis mellifera L.)
Bernhard Kraus*, Eve Gerecke, Robin F. A. Moritz

10:00 8.3. Evolution of antimicrobial peptides in bumble bees
Silvio Erler*, Michael G. Lattorff

10:20 - 10:40 Coffee Break

10:40 8.4. QTL - Mapping of larval Varroa resistance in honeybee drones (Apis mellifera)
Dieter Behrens*, Florence Mougel, Conny Geßner, Qiang Huang, Eva Frey, F. Bernhard Kraus, Peter Rosenkranz, Michel Solignac, Robin F. A. Moritz

11:00 8.5. Honey bee thermal/chemical sensor, AmHsTRPA, reveals neofunctionalization and loss of TRP channel genes
Keigo Kohno, Takaaki Sokabe, Makoto Tominaga, Tatsuhiko Kadowaki*

11:20 8.6. Brood-dependent plasticity in behavioral and molecular circadian rhythms in bees
Guy Bloch*

11:40 8.7. Genotype- environment interactions in Apis mellifera ligustica
Cecilia Costa*, Marco Lodesani, Kaspar Bienefeld

12:00 8.8. Carniolan honeybee (Apis mellfera carnica) conservation in local geographic area
Ales Gregorc*, Per Kryger, Mitja Nakrst, Vesna Lokar

THURSDAY 09:20-12:20 HALL C

Theme V. OPEN SESSIONS: Symposium 14. BeeDoc presentation
Organizer: Robin Moritz

09:20 14.1. Bees in Europe and the Decline of Honeybee Colonies (BEEDOC)
Robin F. A. Moritz*

09:40 14.2. Report of the activities of the BEEDOC 'Diagnostics department'
Dirk C. de Graaf*

10:00 14.3. Bee Doc: novel treatments for the control of honey bee diseases
Robert J. Paxton*

10:20 - 10:40 Coffee Break

10:40 14.4. Bee Doc Prevention Department
Eva Forsgren

11:00 14.5. The structure and function of antimicrobial peptides in defence of honeybee against microbial pathogenes
Katarína Bíliková*, Jozef Simúth

11:20 14.6. The COLOSS network
Peter Neumann*

THURSDAY 09:20-12:20 HALL D

Theme V. OPEN SESSIONS: Symposium 15b. Open Session
Organizer: Meral Kence

09:20 15b.1. Varroa control with Flumethrin after 12 years use in Bulgaria
Kalinka Gurgulova*, Ivanka Zhelyazkova, Vera Popova, Ivan Panchev

09:40 15b.2. Contamination of guttated droplets after dressing of seed with neonicotinoid insecticides: a risk to honey bees?
Jana Reetz*, Klaus Wallner

10:00 15b.3. Age and gender specific release of sex pheromone in the honeybee mite Varroa destructor
Bettina Ziegelmann*, Peter Rosenkranz

10:20 - 10:40 Coffee Break

10:40 15b.4. Pollinator communities of Guaiacum sanctum (Zygophylaceae) in two Caribbean islands
Jose Javier Fumero-Caban*, Lourdes Lastra, Elvia J., Melendez-Ackerman

11:00 15b.5. Public communication of honey bee research
Karin Alton*, Norman L. Carreck, Francis W.L. Ratnieks

11:20 15b.6. Genetic diversity and evolutionary history of honey bee (Apis mellifera L.) populations in Ethiopia
Marina D. Meixner*, Irfan Kandemir, Stefan Fuchs, Walter S. Sheppard

11:40 15b.7. Genetic variation of honeybee(Apis mellifera L.) populations in Iran using RAPD markers
Nasrollah Pirany*, B. Kamrani, A. Hashemy

12:00 15b.8. Bumble bee cryptic taxa discrimination by pheromonal and morphometrics approach
Thibaut De Meulemeester*, Denis Michez, Ahmet Murat Aytekin, Pierre Rasmont

12:20 - 14:00 Lunch Break

THURSDAY 14:00-14:50 CONFERENCE HALL

Plenary Presentation
Muhsin Doğaroğlu
Bee losses and low production due to colony management in Turkey

THURSDAY 14:50-16:30 HALL A

CLOSING SESSION
Organizers: Aykut Kence, Yves LeConte, Tugrul Giray, Meral Kence

EurBee Membership Meeting

Topic 1. Report of Eurbee activities since the last meeting in Belfast 2008

Topic 2. Presentation of a site for the next Eurbee meeting in 2012

Topic 3. Membership fees and membership account

Topic 4. Miscellaneous

16:30 Coffee Break

THURSDAY 17:00-18:00 HALL C

BeeDoc Workshop

Plenary Talks

Consensus Tree and Controversial Polarity
Old and New Approaches to Honey Bee Evolution

Nikolaus Koeniger*, Gudrun Koeniger

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Based on Lindauer's research (1956) on food communication (dance behaviour) of Asian honey bees a phylogenetic tree of *Apis* evolution was constructed: First ancestral *Micrapis* (*A.andreniformis, A.florea)* diverged. Independently and later *Megapis* (*A.dorsata; A.laboriosa)* formed a clade separating from *Medapis* (extant cavity dwelling species). Summarizing the overwhelming evidence documented until today the above consensus tree of *Apis* belongs to one of the best founded results of phylogenetic research.
The Apini together with 3 tribes (Meliponini, Bombini and Euglossini) belong to a monophyletic group, the corbiculate Apoidea (Michener 2000) and cavity nesting is a plesiomorphic character in the 3 other tribes. Therefore, at their origin from corbiculate Apoidea cavity nesting was an ancestral condition in honey bees and the transition to open nesting plays a key role in the discussion of the polarity of *Apis* evolution. The classical concept postulates an early shift to open nesting and positioned ancestral *Micrapis* with horizontal dances near the root of the tree. Later vertical dances occur and ancestral *Megapis* deviated. Then there was a switch back to cavity nesting and medium sized workers resulting in the extant species of *Medapis*. We do not agree with the above evolutionary scenario and here we suggest an alternative polarity.
In *Apis* there are 5 recognized species of cavity nesting bees with medium sized workers (*Medapis*), 2 open nesting species of dwarf bees (*Micrapis*) and at least 2 or even 4 open nesting species of giant bees (*Megapis*). Open nesting species have either large or small workers! And cavity nesting species have medium sized workers! But how has evolution linked "worker size" to "mode of nesting"? Moving to open nesting the ancestral honey bee colony lost the protective cavity walls and the bees had to face an increased predatory pressure. Two alternative routes were open to cope with higher predatory pressure.
The *Micrapis* Syndrome: Many colonies of *A.florea* and *A.andreniformis* have the size of a large leaf and are hiding in between twigs. Whenever a colony is detected by a predator the bees first try to defend. Very often, however, the stings of the dwarf workers are too small and the defence force is too weak. Then the colony takes to wings and absconds. To summarize the small size of its worker seems to be a fundamental feature of the *Micrapis* syndrome like the horizontally directed waggle dance, the unique comb construction around twigs and numerous other characters. The *Megapis* Syndrome: *Megapis* follows an alternative strategy to counter balance the loss of "protective cavity walls". The worker bees are large and their long stings penetrate almost any natural protection. The huge combs are exposed and they rely on their formidable defence and their effective alarm communication. Large Workers as well as the huge open combs hanging under a support and the vertical waggle dance in day light are fundamental parts of the *Megapis* syndrome. The *Medapis* Syndrome: Cavity nesters must communicate via their dance language in the darkness of the cavity. Protected by the walls of the cavities colony defence is focused at the nest entrance and is usually performed by a small group of guard bees. The large majority of the workers are engaged in other activities and generally 50% or more of the workers collect pollen and nectar. So foraging efficiency seems to be a major driving force in worker size of cavity-nesters and have resulted in medium sized, high energy worker bees. In conclusion worker size in honey bees is irrevocably linked to an entire syndrome. The existence of honey bee colonies depends on the functional relations within the syndrome, which is a complex framework of several known and still many unknown biological characters. Each evolutionary change of a single character was integrated in a gradual reconstruction which resulted in the 3 different syndromes which are found today. Accordingly the alternative evolutionary scenario assumes that ancestral cavity nesting bees with medium sized worker bees (ancestral *Medapis*) were most likely in a basal position. From that condition gradual (and arguable!) functional transitions to open-nesting of the *Micrapis* syndrome took place. Further up in the consensus tree the ancestor of *Megapis* separated from ancestral cavity-nesters with medium sized workers *(Medapis)*, which then split in the 5 extant cavity dwelling honey bee species.

Addressing bee decline through the Managed Pollinator CAP - a nationally-coordinated research and education program

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The United States Congress has mandated the U.S. Department of Agriculture to increase funding for research and education directed at reducing honey bee decline. One outcome has been the Managed Pollinator Coordinated Agricultural Project (CAP), a consortium of scientists working in a coordinated manner to reduce institutional redundancy and optimize the discovery and delivery of sustainable management practices to client beekeepers. The CAP activities are organized around four broad objectives: (1) to determine and mitigate causes of bee decline, (2) to deliver pathogen- and disease-resistant bees to the client industry, (3) to improve conservation and management of non-*Apis* pollinators, and (4) to deliver research-based knowledge to client groups.

Varroa mite reproductive biology

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Varroa destructor is the de facto number one enemy of the Western honey bees (*Apis mellifera*) worldwide. Understanding its reproductive biology will help us for its better management. In this presentation, I will present three separate experiments conducted in collaboration with Chinese and French scientists.

Varroa mite reproduction when transferred across species

During 2000-2002 we transferred varroa mites (*V. destructor*, Korean haplotype) from A. mellifera colonies into drone and worker brood of both A. *mellifera* and *A. cerana*. We selected recently sealed (within 6 hours) brood cells as transfer hosts. We obtained phoretic mites from adult workers of *A. mellifera* and transferred them into brood cells with a paint brush after each cell was opened with a small pin. The opening was immediately sealed with melted beeswax after mite introduction. The brood frames were incubated at 35°C for 9 days after which each cell was opened and mite progeny scored. Mites showed high rates of reproduction in all four host groups (>90% for worker and drone brood in both species), but the average number of female offspring was significantly higher in drones than workers in both species. In 2002, we transferred Varroa mites from *A. ceranae* in Xishuangbanna (*V. destructor*, Vietnam haplotype) to *A. mellifera* and found none reproduced on worker brood (N=60) but 97% reproduced on drone brood (N=60). Control transfer to A. cerana drone cells showed 83% of mites able to reproduce. These results suggest that there are haplotype differences in the mite's ability to switch host species.

Large cell size reduces varroa mite reproduction

In the same study, we also accidentally discovered that both *A. cerana* and *A. mellifera* queens lay worker eggs in drone cells in the fall. We took advantage of this and compared the reproductive output of mites on two hosts: workers reared in worker-cells (WW) or workers reared in drone-cells (WD) in *A. mellifera* (Fig. 1). In 2000, mites introduced into WD showed differences from WW in both the percentage of mites that reproduced or the average numbers of female offspring, but the differences were not statistically significant (mean offspring number: $t = 1.6$, $P = 0.12$, % reproduction: $X^2 = 3.59$, $P = 0.058$). This is most likely due to the small sample sizes (N=7 and 13 for WW and WD, respectively). In 2001, with larger samples sizes (N = 47 and 29), we found that only 15% (5 out of 34) of mites reproduced on WD, while 100% (47 out of 47) of them reproduced on WW ($X^2 = 45.7$, $P < 0.0001$). Among these mites that reproduced, they also had less reproductive output: the mean number of female mite offspring was 0.20 + 0.2 (mean + SE) for WD but 2.38 + 0.2 for WW ($t = 3.87$, $P < 0.001$). This is the first study to show that varroa mite reproduction can be affected adversely if the cell size is larger than normal. It is not clear why mites would reproduce less on identical hosts that are housed in larger cells. One possibility is that workers reared in drone cells are fed a different diet by nurses. A second, more plausible, mechanism is that workers spin larger cocoons in drone cells and mites detect this "geographic" change and somehow change their reproductive behavior accordingly.

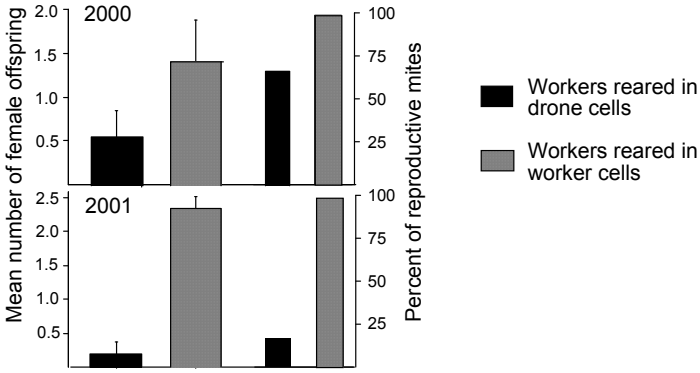

Figure 1. Mean number of female offspring and percent of mites that reproduced on worker brood reared in different cell types. Experiments were conducted in 2000 and 2001 in Beijing, China.

Phoretic host preference of varroa mites

Many studies have shown that *Varroa destructor* prefer nurses, however all studies were performed under laboratory settings. In addition, we do not fully understand why varroa mites prefer nurses. For example, do nurses have different nutrition so that mites reproduce better after feeding on them? Or is it simply because nurses provide the best access to brood stages that are ready to be capped so that mites can invade their host easier? We studied *Varroa destructor* host preference in experimental colonies using *A. mellifera*. We found that the host preference is nurses > newly emerged bees > foragers, with nurses being the most preferred host. To determine why mites prefer nurses, we conducted a second experiment. We reared varroa mites on the three different types of hosts for three days and introduced them into newly capped (<6 hrs) worker brood. Brood was incubated at 34°C and 50% relative humidity. After 9 days we examined the reproductive status and number of offspring of each mite. We discovered that mites reproduced the best when nurses were used as their phoretic hosts, followed by foragers and then newly emerged bees. We now finally understand that varroa mites prefer nurses as phoretic hosts because they give them the highest reproductive potential, possibly due to physiological factors in their hemolymph.

Chemical mimicry of mites to different species of honey bees Previous studies have shown that the varroa mites can mimic the host cuticular hydrocarbons, perhaps enabling them to escape the hygienic behavior of the host honey bees. We transferred varroa from *A. cerana* to *A. mellifera*, and vice versa and studied their chemical profiles. We discovered that regardless of their prior host (whether *A. cerana*, or *A. mellifera*), varroa mites showed similar hydrocarbon profiles to their second host (the transferred to host). This is true for both mites originally from A. cerana (Korea 2 haplotype), and mites originally from A. mellifera (Korea 3). We therefore demonstrate that the varroa mites are able to mimic the host cuticular hydrocarbons, even when they are transferred artificially across different host species. This remarkable ability to mimic host chemically may explain their recent host-shifting from *A. cerana* to *A. mellifera.*

Honeybee colony losses and the decline of beekeeping

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Over the last years, high winter losses of managed honeybee, *Apis mellifera*, colonies have been reported from many countries in the Northern hemisphere, but the underlying reasons remain partly understood, thereby preventing efficient mitigation strategies. *Varroa destructor* certainly plays a key role but cannot explain the current major losses alone. Indeed, dying colonies show a wide range of symptoms (incl. CCD (= Colony Collapse Disorder) and no single factor emerged as the definitive cause. Instead, it seems as if interactions between factors (e.g. pathogens, poisoning, nutrition and miss management) rather than conventional monocausal causes are the most likely explanation. Such interactions are inevitable in Europe due to the ubiquitous mite *V. destructor* and viruses (e.g. DWV). Finally, novel factors such as *Nosema ceranae* further complicate the picture not only for scientists but also for apiculturists, rendering beekeeping a more laborious work and a less rewarding hobby. It is therefore not surprising that European beekeeping is in decline, creating demand not only for a better understanding of honeybee health, but also for adequate socioeconomic and political solutions. Two respective FP7 consortia (BEE DOC = Bees in EuropE and the Decline Of Colonies) and STEP = Status and Trends in European Pollinators) and a COST Action have been launched (COLOSS = Prevention of honeybee Colony LOSSes), but more sustainable approaches at both national and EU level seem necessary to limit colony losses and the decline of apiculture.

Reproductive conflicts honeybees: how to kill a queen and get away with it

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The queen typically monopolizes female reproduction in the honeybee colony (*Apis mellifera*). A suite of queen and brood pheromones prevents worker reproduction and in addition the worker police most efficiently remove worker laid eggs from the brood combs. Nevertheless, there are many occasions where workers gain direct fitness. This is common under queenless conditions but also occurs in queen right colonies. Although queen control is strong it is not complete and there is wide scenario under which laying workers develop into so called pseudoqueens. This is most extreme in the Cape Honeybee, *A. m. capensis*, where laying workers can invade queenright colonies, kill the queen and take over the colony as a social parasite. But not only workers also queens themselves can embark on a social parasitic life history trajectory. I will highlight the various tactics on the battle fields of intracolonial competition selection, taking the analysis from a colony level down to the molecular switch which controls parasitic behaviour in workers

Pheromone involvement in reproductive competition in the bumblebee Bombus terrestris

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Bombus terrestris forms monogyne colonies headed by a singly inseminated queen. Kin selection predicts therefore that queens and workers compete for male production. Inclusive fitness and the annual life history of colonies dictate a narrow window for worker reproduction, before season ends but after gynes have been produced. Accordingly, colony development follows an ergonomic phase typified by queen reproductive monopoly and harmonious worker behavior, and a competition phase typified by worker reproduction and all-out aggressive interactions. While unequivocal evidence for coercive pheromone-inhibition of worker reproduction still wants, behavioral evidence suggest that queens may have a suite of pheromones that affect both worker behavior and caste determination.
Chemical analysis of queen and worker exocrine glands revealed qualitative differences in several exocrine glands. Among these, pheromone caste specificity of Dufour's gland exudates in *B. terrestris* is unique in that workers possess in addition to typical to all castes hydrocarbons, a series of octyl esters that are absent in queens and disappear when workers undergo transition from sterility to fertility. Behavioral and chemical analyses revealed that the workers that contain the octyl esters are less molested by dominant nestmates during the turbulent competition phase. We therefore suggest that these esters comprise sterility signal and their function is to appease dominant females they may encounter. Such a signal facilitates the reinstatement of a reproductive division of labor and through that insures good colony reproductive output, and thence higher inclusive fitness to all colony members.

Mechanisms For Learning And Memory Of Complex Floral Odors

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Flower odors are highly variable combinations of several volatile components. No two flowers smell exactly alike, even examples from the same species and cultivar. Honey bees must therefore establish if a newly encountered flower is similar *enough* to a previous rewarded one, turning foraging decisions into a fine tuned generalization-discrimination problem. Sensory and neural systems must provide mechanisms for very precise odor recognition, allowing perceptual stability (i.e. generalization to prevent all experiences from being independent and novel). We hypothesize that experience with odors tunes sensory processing and thereby improves odor recognition and classification of newly encountered flowers. To test this hypothesis, we have designed artificial floral blends that mimic the components, proportions and variability of 2 different natural varieties of snapdragon flowers. All designed blends share the same components. But they can be differentiated based on the relative concentration of the components, which were more similar within than between cultivars. We trained restrained honey bees using the proboscis extension response paradigm (PER). Bees were differentially conditioned using examples of both cultivars. After training we tested the ability to recognize a new example from each cultivar. The duration of PER was lower and the latency longer when an example of the non-rewarded cultivar was offered to the trained bee. We also evaluated how early sensory processing in the honey bee brain can be tuned to enable this fine discrimination. We measured odor induced activity patterns in Projection Neurons of the Antennal Lobe by calcium imaging. Consistent with behavior, results suggest that the neural network in the AL is tuned via differential conditioning to decorrelate mixtures representing different floral varieties. At least two different biogenic amines are involved in this plasticity. Experience-dependent plasticity at the level of the Antennal Lobe may help animals categorize a newly encountered flower as belonging to a class related to nectar or pollen rewards.

Bee losses and low production due to colony management in Turkey

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The position and geographic condition of Turkey create a very rich biodiversity for honeybees and floral sources. There are many races and ecotypes of bees which easily adapt to the various ecologic conditions and very rich floral sources suplying nectar and polen to the honeybees lasting all year long.
Wild flowers in many different altitudes, some agricultural sources and forest tree flowers are also nectar sources for honey production of the country. In addition to these floral sources there are also very strong honeydew sources on pine tree forests in the Southwest region of the country. These sources produce one third of country's honey production and represents 92 % of the wolds' honeydew honey production.
In Turkey, the 5 million colonies in existance ranks second in the world and their 74 thousands ton honey production is ranked 5th. The countries production apparently appears unproductive but with potential to produce more. The mean honey production of Turkey is ranged 15-17 kg which is very low when compared to some other countries who produce about three times more. Turkey's mean production is also under the world level.
There are many reasons cause this failure and also these reasons increase colony loses. These reasons and results are mostly attributed to the many diseases and predators by beekeepers. Of course these all couses may generate loses but in many manners loses may caused from beekeeper's own applications and even some from their measurements. Therefore in most circumstances management methods take the first place of bee loses as we assume happening in Turkey. Low population levels; less productivity, incorrect usage of breed lines, races, ecotypes and floral sources; rearing and using low quality queens; high swarming tendency; none utilising modern equipment and some other reasons are compose of bee loses management reasons in Turkey.

ORAL PRESENTATIONS

Theme I. BEE LOSSES: Symposium 1. Pests, Pathogens and bee loss I: focusing on Varroa and viruses

Varroa destructor mite reproduction in honey bee colonies surviving mite infestation in Sweden and France

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Non-managed populations of the European honey bee documented to have survived miteinfestations for a substantial time without mite control treatment, suggest that some level oftolerance to the pest and possibly even a sustainable host-parasite relationship has beenestablished between A. mellifera and V. Destructor in these populations. In a well documented population of surviving honey bee colonies in Sweden, an exploratory investigation of colony level traits linked to Varroa tolerance was conducted in order to identify characteristics that may be responsible for their survival in spite of the mite infestations. Significant results in reduced mite reproductive success in the Swedish population prompted an investigation of this trait in a honey bee population in France also reported to have survived mite infestations without control treatments for over a decade. Our data suggest that colony level adaptive traits may limit mite population growth by suppressing the mite reproductive success, but that the selection parameters influencing the development of this trait and the echanisms behind it may be different in different population. A more detailed investigation in the Swedish population revealed hygienic behaviour, grooming behaviour and brood attractivity to be not significantly different parameters of tolerance between the surviving population and control colonies. Colony size and brood amounts were however significantly reduced in the surviving colonies in Sweden which may be an adaptation by the colony to limit mite reproduction opportunities. Virus infection dynamics in the Swedish surviving population will also be discussed.

The deformed Wing Virus (DWV) is poorly pathogenic for queen honey bees (*Apis mellifera* L.)

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[3]Montpellier SupAgro, Montpellier, France
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The economical value of bee colonies is tightly linked to queen health. Because of their relatively long lifespan, queens are more exposed than worker bees to the different toxins and pathogens that contaminate their food. The abundance of viral particles in bee products like pollen, honey and royal jelly raises the question of whether these viruses affect the physiology of queens. We therefore checked for the presence of 10 honey bee viruses in different queen samples collected in France and found that DWV was most prevalent in mated queens. DWV RNA was detected in various queen organs, especially in fat body and in the upper part of the ovary where the oocytes differentiate. The DWV titres recorded in ovaries were found correlated with higher titres in abdomens. However no impact of these infections on the queen fitness could be demonstrated. Likewise, we could not show a clear effect of DWV infection on vitellogenin or vitellogenin receptor genes expression in ovaries and abdomen. Put together our data suggest that DWV is poorly pathogenic for queen honey bees.

Involvement of deformed Wing Virus (DWV) and Varroa destructor virus (VaDV) in the deformed wing syndrome of the honey bee

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Recently we performed a country- wide screen for viruses affecting honey bees in Israeli apiaries that revealed a high incidence of the Deformed wing virus (DWV) and the *Varroa destructor* virus (VaDV). In order to understand better the relationship between the presence of the above viruses and the deformed wing pathology, we performed qualitative and quantitative analysis of DWV and VaDV replication in individual bees from apiaries with overt infections, utilizing RT-PCR and quantitative Real-Time PCR. Also, we followed up the presence of the above viruses by immunoblot analysis of the viral capsids in the infected bees. Our data indicate that both viruses may be able to induce the deformed wing pathology depending upon their ability to replicate efficiently in the bee host.

Impact of virus infections on vitellogenin expression: mechanism contributing to colony losses?

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Over the last years, high winter losses of managed honey bee, *Apis mellifera*, colonies have been reported from the Northern hemisphere, but the underlying mechanisms remain partly understood. Expression of the vitellogenin gene (Vg) is an indicator of the physiological state of workers and maybe influenced by virus infections, thereby constituting a mechanism for colony weakening and ultimately losses. Here we monitored 29 *A. m. carnica* colonies from September 2007 to April 2008 and sampled pooled (N=100 each colony) and single (N=558) workers in summer, fall and winter. Then, the pooled and single workers were analyzed for Vg expression and infections with Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV). Our data show significant correlations between Vg expression and both DWV and ABPV infection levels in pooled and single workers, suggesting that virus infections can interfere with the physiological state of the workers. Moreover, Vg expression was significantly higher in bees from colonies, which were dying compared to surviving ones. The observed correlations propose another mechanism contributing to honeybee colony losses and provide Vg expression as a predictive marker for colony death.

Remebee - RNAi based product-line targeting major culprits in Beekeeping

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*E-mail: gal@beeologics.com

Three new RNAi-based applications for honeybee are presented by Beeologics for specific, safe and efficient solutions for the major culprit and pathogens including: viruses, *Nosema* and *Varroa* mite. The use of naturally occurring and target specific bio-technology called RNAi, has brought a new hope for the beekeeping industry after years of colony loss.
Beeologics has established a simple and relatively inexpensive procedure to produce large quantities of dsRNA homologous to target pest or pathogen sequences. Remebee™ is the company's leading RNAi product produced in-vitro which is homologous to honeybee viral sequences. The exogenously supplied Remebee mimics the natural mechanism involved in silencing viral replication, within the honeybee cells. In large scale field trials, the gene silencing mechanism induced by Remebee that was fed to the bees had shown to be highly effective in preventing honeybee mortality from the *Israel Acute Paralysis Virus* (IAPV).
The Colony Collapse Disorder (CCD) phenomenon is still not fully understood or agreed upon; however, there is a strong consensus that pathogens and pests are major contributing factors to Colony losses. Viruses, microsporedia such as the *Nosema Ceranea* and the *Varroa mite* are considered the top three pathogenic contributors to the phenomena. Beeologics has developed a generic technology platform which is utilized to introduce a full RNAi product-line targeting all three culprits.
The uniqueness of RNAi enables scientists to develop silencing strategies with new organisms. Basic elements of the new products' design including: mechanism of action proof of action, regulation and ongoing development will be discussed.

Application of Porous Ceramics in Delivering Lemongrass Oil to Control Mites and Bee Pathogens

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American foulbrood, European foulbrood, Chalkbrood diseases and the parasitic mites are serious problems for beekeepers. These problems have led to the death of bee larvae and adults, and ultimately, in many cases, severe colony loss. The common method to treat these problems is the application of some chemical and antibiotics, however, such treatments are not generally acceptable, due to serious concern of chemical residues remained in honey bee products. The alternative approach using essential oils was studied in vitro. Lemon grass oil has been found to be the most effective agent against American foulbrood, European foulbrood, Chalkbrood pathogens and the parasitic mites. In this study we pursue an effective means to deliver the volatile oils by using porous ceramic materials as supporting media. In field trials, we used porous ceramics prepared by the mixture of diatomaceous earth and activated charcoal as the main starting materials. The porous ceramics which showed the maximum water absorption of 75.96% was chosen as the supporting media for lemon grass oil to combat the diseases and parasitic mites

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Negative side effects of viruses and Varroa destructor on honey bee colony survival in Belgium

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Since 1999, Belgian beekeepers observe abnormal increases in overwintering mortality in honey bees, *Apis mellifera* L. Viral infections are often cited as a potential cause of their collapse. There is increasing evidence that the global spread of *Varroa destructor* has resulted in a significant change in the type and prevalence of viruses causing mortality in honey bee colonies. We report here the first survey of the prevalence of five bee RNA viruses, observed during a large-scale sampling of adults covering the winter period of 2006 – 2007 in Belgium apiaries. The samples were analyzed by RT-PCR for virus identification. Black queen cell virus (BQCV) was found at least once in 75% of the apiaries, chronic bee paralysis virus (CBPV) and sacbrood virus (SBV) were found in 69% of the apiaries, deformed wing virus (DWV) was found in 64% of the apiaries, and acute bee paralysis virus (ABPV) was found in 8% of the apiaries. Finally, we evaluated the potential correlation between mortality rate and both the presence of these viruses and phoretic varroas. A negative effect of viruses co-infection and *V. destructor* was observed on colony survivorship.

Relationships between integrated viral sequences and bee behaviour and horizontal transfer of RNAi in bees

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Restudying a case where bee disappearance was not correlated to the presence of IAPV was re-examined for the presence of integrated viral sequences in the genome. A strong correlation was obtained between the presence of integrated
sequences in the genome and bee disappearance. In addition we found the almost 100% of *Varroa destructer* carry an IAPV sequence in their genome.
We also report on the stability of dsRNA (RNAi) in the honeybee body and its vertical transmission to varroa mites and back to the bees, enabling gene manipulation of one organism by treating the other one.The possible significance of genomic integration and RNAi transfer will be discussed.

Theme II. DIVERSITY AND CONSERVATION:
Symposium 5. Diversity of bees

Revealing molecular biodiversity within East-European honey bees

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Honey bee (*Apis mellifera* L) populations show morphological, ecological and ethological differences, so that the different subspecies have been grouped through morphometric and molecular analyses in five evolutionary lineages correlated to different distribution areas: Africa (A lineage), Western Europe (M lineage), Eastern Europe (C lineage), Near East (O) and Ethiopia (Y). The C lineage includes four subspecies: *A. m. ligustica* (Italy), *A. m. cecropia* (Southern Greece), *A. m. macedonica* (Bulgary and Northern Greece) and *A. m. carnica* (Balkan countries). Different ecotypes have been described within these subspecies based on the morphometric variability, climate and habitat variation. The aim of the present study is to evaluate the genetic diversity (variation of the mitochondrial $tRNA^{leu}$-cox2 intergenic region) and population structure (eleven microsatellite *loci*) of honey bee populations from Eastern Europe, focusing in these previously described ecotypes in order to discriminate among them. The detailed knowledge of the genetic diversity and the population structure of Eastern Europe honey bees will allow carrying out correctly and in an effective way different management plans and conservation policies. Likewise it will allow a suitable analysis of the introgression events in populations where honey bee queens from these countries are being introduced.

Barcoding: It is a useful tool for the discrimination of honey bee, *A. mellifera* populations?

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The discrimination of bee's subspecies is very important, but not yet standardised. Different approaches are applied in several countries, based on different methodology: classical morphometrics (but the characters that are measured are not always the same: cubital index is the most common character that is used), geometric morphometrics (3 different methods), molecular markers (isoenzymes, mitochondrial DNA, microsatellites etc). There is the necessity to develop a common protocol for the certification of honey bee genetic origin. The Barcoding method may be a useful tool. Research is in progress using sequencing analysis of mitochondrial cytochrome c oxidase subunit I segment (COI) based on Barcoding primers, on samples of bee populations originating from different European subspecies. PCR products are purified and individual sequences are determined via automated sequencing. Multiple-sequence alignments are done with CLUSTALW2 and for data processing, the packages MEGA 4 and BioEdit 7.0.9.0 are applied. The sequencing of the Barcoding mtDNA gene studied produce an alignment of about 650 bps. Preliminary results show that honey bee populations can be grouped into different haplotypes. The Barcode method maybe suitable for the discrimination of *A. mellifera* populations based on maternal inheritance. Paternal introgression calls for other markers to be applied.

A Europe-wide experimental approach to reveal genotype-environment interactions

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The existing subspecies and ecotypes of honey bees in Europe represent an important resource for breeding of disease and stress resistant strains. The objectives of our experiment are to develop and test internationally recognized criteria for vitality, to establish standardized methods to assess honey bee colonies based on these criteria and to investigate the role of interactions between genetic diversity and environment on honey bee colony vitality. A common experiment has been set up, comprising a total of 670 colonies from 17 different genetic origins in 16 locations across Europe. Each location contains the local strain of bees together with at least two “foreign” origins. The colonies will be managed and evaluated according to a standard protocol that is used by all participants. Chemical treatment against mites is precluded, except when a colony is in immediate danger of collapsing. Apart from traditional criteria of selection, additional parameters related to vitality, such as mite infestation level, hygienic behaviour and the occurrence of other diseases are being continuously evaluated. From the results of the experiment we expect insights into the interactions between genetic origin and local adaptation, and contributions to our understanding of interactions within the bee-mite-environment system. In addition, the participants will have established and evaluated a standardized test methodology to estimate the vitality of bee colonies under varying environmental conditions across Europe and thus contribute to the conservation of locally adapted strains of bees. .

New data about allozyme variability of Bulgarian honey bees

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The genetic variability of honey bee populations from twenty four different provinces from Bulgaria and populations of *A. m. carnica* from Serbia, *A. m. caucasica* from Poland, *A. m. ligustica* (imported in Bulgaria from Italy) and *A. m. macedonica* from FYR of Macedonia has been studied using isoenzymic analysis of six enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 loci. All of studied loci were found to be polymorphic in most of the populations. Four alleles were detected at Mdh-1 locus (Mdh 65, Mdh 80, Mdh 100 and Mdh 125). The allele Mdh 125 was found to be present only in “carnica” population. Four alleles were detected at Me locus (Me 90, Me 100, Me 106 and Me 115) – Me 100 was fixed in one of Bulgarian populations and Me 115 was found to be present in only one of these populations. Est-3 locus was polymorphic with six alleles - Est 80, Est 88, Est 94, Est 100, Est 105 and Est 118, one of which (Est 100) was fixed in two of Bulgarian populations. Three alleles were detected at Alp locus (Alp 80, Alp 90 and Alp 100), four alleles - at Pgm locus (Pgm 80, Pgm 100, Pgm 114 and Pgm 125) and three alleles - at Hk locus (Hk 87, Hk 100 and Hk 110). Pgm 100 was fixed in one of the Bulgarian populations. Pgm 80 was detected in “caucasica” honey bees. The calculated percentage of polymorphic loci ranged between 50% and 100%. The observed and expected heterozygosities (H_o and H_e) ranged from 0.142 to 0.253 and 0.219 to 0.296, respectively. Allele frequencies of all loci were used to estimate Nei’s (1972) genetic distance, which was found to range between 0.001 and 0.078. Neighbor-Joining phylogenetic tree and UPGMA dendrogram were obtained by genetic distance matrix methods. *A. m. carnica*, *A. m. caucasica* and *A. m. ligustica* were clustered separately of *A. m. macedonica* and all Bulgarian populations studied which formed different branch. The result obtained in this study show that Bulgarian honey bees genetically are much closer to *A. m. macedonica* than to *A. m. carnica*.

Intracolony selection of the Dark European honey bee for disease Resistance

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In recent years, extensive losses of honey bee colonies have been reported from many countries worldwide, and many possible causes have been suggested and explored. Whilst the causes may vary between countries, it is generally agreed that the phenomenon is multi factorial in nature. A key factor in these colony losses is, however, undoubtedly the parasitic mite *Varroa destructor*, which is now present in all major countries apart from Australia. One reason for continuing losses is that in many areas *V. destructor* has become resistant to the synthetic pyrethroid acaricides which were previously successfully used to control it. Early studies in the USA showed that certain strains of honey bees exhibit so called "hygienic behaviour", which enables them to resist bacterial and fungal brood diseases, and more recently hygienic behaviour has also been shown to confer resistance to *V. destructor*. We are using the novel technique of intracolony selection to enhance hygienic behaviour in the British native honey bee *Apis mellifera mellifera*, with the aim of reducing the reliance on chemical acaricides in bee colonies.

Biodiversity of the honeybee population from La Palma (Canary Islands, Spain) and influence of the conservation program on a natural mating area

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Present biodiversity of *Apis mellifera* populations from La Palma (Canary Islands, Spain) has been analysed with molecular data obtained of the variation of the mitochondrial $tRNA^{leu}$-cox2 intergenic region and five microsatellite *loci*. This honeybee showed mainly characteristic haplotypes that included these populations within the Atlantic sublineage (A_{III}) of the evolutionary lineage of African subspecies, but introduction of managed foreign subspecies has been also detected. The preservation and dissemination of black honeybee queens among beekeepers on La Palma have increased the genetic diversity of the honeybee population on the island, and therefore this population could become a useful source of black Canarian honeybees for other Canary Islands. In order to evaluate the effects of the conservation program to protect the local Canarian black honeybee established since 2001, the molecular variability was also estimated in colonies from a natural mating area located at the north of the island in two surveys from 1998 and 2006. Mitochondrial variability has significantly changed in this area whereas with microsatellite data this difference is not detected.

Genetic Diversity of the honeybee populations (*Apis mellifera* L.) from Turkey and Iran based on 20 microsatellites

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The genetic variation of honeybees collected in 75 apiaries from 5 different localities (Ardahan, Posof, Van, Hakkari and Muş) in Eastern part of Turkey and 20 apiaries from 5 different localities in (Tabriz, Maragheh, Urumieh, Ziveh and Piranshar) Western part of Iran was analyzed using 20 polymorphic microsatellites. The overall genetic diversities were ranged between 0.52 (Posof) to 0.58 (Van). The genetic distances were calculated as 0.04 between Van and Muş and 0.28 between Hakkari and Posof. According to microsatellite allele frequencies, Turkey and Iran honeybee populations are mainly clustered into two different groups by NJ phylogenetic analysis.

Functionally different sex-alleles in honeybee populations: a tool to determine allele frequencies

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In honeybees sex is determined by the allelic condition of a single gene, the sex-locus. Heterozygotes develop into females and hemizygotes as well as homozygotes develop into males. Homozygous males are completely diploid and get cannibalized by workers constituting an enormous fitness loss. This leads to balancing selection at the sex-locus favouring rare alleles which might rarely form homozygotes. The responsible gene has been identified by positional cloning (*complementary sex determiner, csd*). Sequences between individuals and populations differ, but unfortunately the functional unit responsible for allelic differences has not been identified.
We developed a method utilizing tightly linked microsatellite markers that allow for tagging an allele by its associated allelic pattern. Patterns are analysed in workers that, by definition, carry functionally different alleles. The number and frequencies of sex alleles within a closed honeybee population maintained on the island Schiermonnikoog (Netherlands) was determined. Microsatellite profiles were obtained for 58 chromosomal sets of queens and 100 chromosomal sets of drones. 15 functionally different sex alleles were found with an effective number of alleles of 11.1. This indicates that the number of matched matings is about 18%, although no severe signs of diploid male production have been observed. The allele frequency distributions of queens and males (representing the preceding generation) are highly correlated indicating that genetic drift might has a minor impact in this population. An analysis of genotypic combinations showed a highly significant correlation between observed and expected genotypes, indicating a robust and comprehensive sampling of sex alleles.

Theme III. BEE BIOLOGY and ECOLOGY Symposium 7. Bees and Pollination
Organizers: Pierre Rasmont and Murat Aytekin

The role of bees as pollinators for *Vicia faba* in the UK

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Bees provide an important ecosystem service by pollinating many agricultural crops and wild plants. Commercially managed honeybees have been introduced across the World for crop pollination over the centuries, but in recent decades have suffered major declines. Wild bees have been proposed as an insurance policy against ongoing honeybee declines, but have also shown declines in the UK in recent decades and as important pollinators of many wild plant species are of direct conservation concern. However, little is known about how much wild pollinators actually contribute to pollination in many systems, or how land management practices may effect the populations of these important pollinators. Field beans (*Vicia faba)* are an important agricultural crop in the UK and although reported to be wind pollinated it is believed the yield can be increased by the presence of pollinating insects. We placed field bean plants in the margins of cereal fields on organic and conventional farms to investigate the role of bees for seed set of *Vicia faba* and the influences of agricultural and landscape factors on these pollination services.

A Biogeographical Analysis of the Genus Evylaeus Robertson (Hymenoptera: Halictidae) of Turkey

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Evylaeus Robertson is the largest genus of the family Halictidae (Apoidea: Hymenoptera). It contains nearly 350 currently recognized species in Palaearctic Region. However data on the West Palaearctic distribution of these species is scanty. For this reason, checklists of the most prominent bee databases and the literature were analyzed to figure out the West Palaearctic distribution of the genus. The species composition of Middle Asia, Caucasia, North Africa, Europe and Turkey were compared. Besides, Mediterranean area of West Palaearctic which is one of the main biodiversity hotspot regions of the world was discussed according to the occurrence of these species. On the other hand, it was found that *Evylaeus* fauna of Turkey is one of the richest fauna with 119 species. Moreover the information on the flower choices of the members of the genus was also discussed. In conclusion, this study reviewed the general status of the genus in Turkey and provides a base for future taxonomic studies.

Evolution of host-plant specialization in wild bees, physiological or behavioral constraint ?

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Interactions between flowering plants and wild bees are higly diverse. Some bee species are specialized in narrow range of ressources (i.e. oligolectic species) while other species forage on numerous plant families (i.e. polylectic species). Some clades of wild bees like the Melittidae include only oligolectic bees. Ancestral host-plant and specialist behavior seem both highly inherited. However some rare "host-plant shifts" occured during evolution inside clades. The origin and the mechanism of these host-plant shifts remain misunderstood. Similar morphology of alternative host-plants could make the shift easier but the need of particular chemicals (sterol, protein, ...) in pollen could reduce the range of suitable hosts. We present our first results on chemical composition of host-plant pollen..

Effects of hedge connectivity and adjacent crop fields on the pollination of shrub species

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The importance of hedgerow-forest connectivity and adjacent crops, in particular the effects of the mass-flowering oilseed rape was investigated on bee and hoverfly abundance and pollination success in Göttingen, Germany, 2009. Six replicates of forests edges, hedges connected to forests and isolated hedgerows were selected next to winter cereal fields and six next to oilseed rape fields. Pollinator abundance was investigated on blackthorn (*Prunus spinosa*), hawthorn (*Crataegus* sp.) and hip (*Rosa canina*) during the species' flowering time. Bees and hoverflies were counted and sampled during one observation event on each plant. The effectiveness of pollination was measured on previously marked branches by determining fruit set and fruit weight. To test for pollination limitation, 2 branches/plants were bagged to exclude pollinators before the beginning of the flowering period and the fruit set was compared between the covered and open branches. There were significantly more pollinators on blackthorn in the forest edges than in the isolated and connected hedges, however, its early and short flowering period during rainy weather resulted in almost no fruits. Connectivity had no effect on the abundance of pollinators in case of hawthorn and hip, however, it was enhanced by the higher amount of hawthorn and hip flowers and the more pollinators had positive effect on the pollination success of hawthorn. More pollinators were found on hip next to oilseed rape fields than bordered by winter cereal fields. The fruit weight of hip was significant higher in case of the open branches than at the covered ones.

Bumblebee diversity and flower visits on experimental long-term set-asides in southern Finland

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Farmland biodiversity can be enhanced either by managing existing habitats or creating new ones, such as long-term set-asides, on arable land. We studied the diversity and flower visits of bumblebees (*Bombus*) during six years of set-aside succession. Additional data was collected on surrounding, untreated field margins. This allowed us to track the colonization process of the set-asides by the bumblebees and a comparison group, the butterflies. The experiment itself consisted of two treatments: seed mixture (competitive, less competitive or diverse mixture) and mowing (annual mowing or no mowing). The diverse mixture with nectar and pollen plants proved superior in terms of both bumblebee species richness and abundance. In addition, the less competitive mixture outperformed the standard mixture. Systematically more bumblebee species and individuals were observed on the mown plots during the last years of the experiment. The main forage plants were *Phacelia tanacetifolia* during the first, *Vicia villosa* during the second and *Vicia cracca*, *Centaurea jacea* and *Lathyrus pratensis* during the last four years of the experiment. *V. cracca* and *L. pratensis* established through natural succession, whereas the other three species were sown in the diverse mixture. Visits to *P. tanacetifolia* were clearly dominated by *B. lucorum*, 94 % of the visitors belonging to this short-tongued species complex. The other four species attracted a more diverse sample of bumblebees, about 34 % of the visitors to *V. villosa*, *V.cracca* and *L. pratensis* being long-tongued *B. distinguendus*. Based on our results, we give recommendations in terms of set-aside establishment and management.

Temporal dynamics of the male effective population size in bumblebees (Hymenoptera:Apidae)

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Bumblebees are of major ecological and economic importance. As in all social Hymenoptera, sociality and haplodiploidy leads to a generally reduced effective population size (N_e) associated to reduced population fitness. Using microsatellites on a large field sample of drones and workers of *Bombus terrestris* and *B. lapidarius* we tested two hypothetical scenarios of drone dispersal potentially increasing effective population size: 1) genetically distinct drone-cohorts sequentially pass through an area or 2) Drones belong to one large temporarily unstructured population with extended mating flight range. We used two different colony assignment approaches, deriving natal queen genotypes either from weekly separate drone sub-samples or from the overall sample, referring to the two Hypotheses. The majority of the drones in our sample-area originate from colonies farther away from the sampling location than the workers foraging range. Our results indicate a clear genetic differentiation between local, worker contributing, and foreign, only drone contributing colonies leading to an increase of the populations gene-pool. However, analysis of colony assignment variance and temporal distribution of drone-contributing colonies suggested Hypothesis 2 to be the more parsimonious one. Though queen dispersal remains to be studies, we argue that N_e in bumblebees may be increased through extended male mating flight ranges, estimated with 10.54 km^2 and 15.98 km^2, *B. terrestris* and *B. lapidarius*, respectively, almost doubling local colonies foraging range in both species. Accordingly, population genetic characterizations indicate strong, genetically highly diverse populations with no signs of inbreeding.

Genetic colony structure and male production in the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

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Cooperation and conflict in social insects is closely linked to the genetic colony structure and mating frequency. Kin selection theory predicts, based on the different degrees of relatedness, conflict over the production of males between the workers and the queen. In bumblebees (*Bombus*) the majority of species are single mated and male production seems to be dominated by the queen in the species studied so far. However these studies were conducted with only a few temperate climate species. Here we used the neotropical bumblebee *B. wilmattae*, to determine genetic colony structure and investigate the outcome of the queen worker conflict over male production. A total of 204 workers from nine colonies were genotyped with up to nine microsatellite markers to infer the queen genotype and the number of males each queen had mated with. Two of the nine queens were double mated and seven mated only once, resulting in an average mating frequency of 1.22 ± 0.44. In the colonies with the two double mated queens, the distribution of the patrilines was not even, resulting in effective mating frequencies of 1.66 and 1.70 respectively and average relatedness of 0.54 ± 0.01. The workers clearly dominated production of males with an average percentage of 84.9 % and no indication for worker policing could be found in the double mated colonies.

Theme I. BEE LOSSES:
Symposium 2. Pests, Pathogens and bee loss II: focusing on Nosema and viruses

Young and vulnerable-chalkbrood infection experiments in honey bee larvae of variable age

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It is known for American foulbrood and sacbrood that young larvae are more susceptible that older larvae, whereas contradicting reports of age dependent susceptibility are given for chalkbrood. 3-4 days old larvae are by some authors said to be most susceptible to chalkbrood, while other authors state that 1-2 days old larvae are also very susceptible. We investigated the age dependent susceptibility in controlled exposure bioassays. Groups of *in vitro* reared honeybee larvae of various ages were fed with *Ascosphaera apis* spores, the causal agent of chalkbrood, and mortality was recorded daily. Exposed to the same dose of chalkbrood spores the mortality was significantly higher among younger larvae compared to older larvae, thus documenting a clear age dependent susceptibility. In addition, the speed of infection was higher in younger larvae and most of them were actually killed by the fungus before reaching the last larval instars, the larval stage which will normally be capped by worker bees. Hygienic behaviour, uncapping and removal of mummies, is strongly induced by the presence of chalkbrood. Uncapped diseased and dead larvae will most probably quickly be detected, eaten or removed by the worker bees. Chalkbrood infected young larvae will therefore not be detected by beekeepers. Our findings will be discussed in light of the disease cycle and detection of clinical symptoms.

Interaction between Nosema spp. and a neonicotinoid affect honey bees at the individual and social level

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Single factors, like parasites and pesticides, have been linked to honeybee losses but none of them have been identified as the main cause. At present, it is believed that a combination of these agents is more likely to contribute to the current honeybee decline. Consequently, we tested the hypotheses describing honeybee losses as a multifactorial syndrome by investigating the interactive effects between *Nosema* microsporidia and imidacloprid (neonicotinoid) on different parameters of honeybee health. We measured mortality, individual and social immunity and pheromone production. Bees were artificially infected with *Nosema* and chronically exposed to imidacloprid. We demonstrated that the interaction between *Nosema* and imidacloprid at doses encountered in the environment significantly weakened honeybees. While no effect was observed on individual immunity, the combination of both agents caused the highest mortality rates and significantly decreased glucose oxidase (GOX) activity, a parameter of social immunity. In addition, *Nosema* infection significantly increased ethyl oleate (EO) production, a worker primer pheromone, above levels naturally found in bees. However, imidacloprid alone or in combination with *Nosema* did not affect EO production. Since GOX is essential for sterilizing the colony and brood food, our results suggest in the long-term a higher susceptibility of the colony to pathogens. In addition, as EO is involved in the regulation of division of labor between workers, one could also expect in the long-term a disturbance of social communication that may endanger the whole colony homoeostasis. Further investigations of these interactions in the field are needed to confirm these long-term effects.

Beepath, a newborn association of apidology

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In Italy, many specialists are working in bee related subjects: researchers , veterinarians, beekeepers.The need to see those groups collaborating efficiently led to create the BeePath association in January 2010. At the round table "Vets and Apimondia", held at the 41st Apimondia Congress, an International coordination between the veterinarians involved in the field of apiculture was established. Notwithstanding this International effort, the situation in several European countries is far from optimal. In Italy, for instance, the possibility for a students to receive an apidological education at the Faculties of Veterinary is largely insufficient (one course is provisionally active at the Bologna University in 2010), whereas post degree master courses are occasionally held (only the Pisa University holds masters in "Apidology and bee pathology" intended for vets). BeePath tries to cover integration and education needs for vets and for other specialists related to apiculture and to apidology,with the goals to organize meetings, to be a reference at Institutional level, to improve the environment and to promote the honey bee wellbeing, according to a wide collaborative approach. To this end, a scientific and a sanitary commission were created within the association.

Prevalence of pathogens and tracheal mite in honey bees in Japan

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A shortage of honey bees for pollination in Japan occurred in the winter of 2008. Japan's suspension of imports of queen bees from Australia in 2007, the effects of agrochemicals and various pathogens on honey bees are thought to have contributed to the shortage. To identify the major causes of honey bee decline in Japan, we conducted the first nation wide survey of bee pathogens and tracheal mite in both European and native Japanese honey bee populations. We have found that tracheal mite and various pathogens (viruses, bacteria, fungi, and microsporidia) are present in *Apis mellifera,* and some of them have infested *A. cerana japonica.* The current state of honey bee health conditions in Japan will be discussed.

What has Nosema got to do with losses? Monitoring both Nosema species in the UK

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In recent years scientists have become aware of a second *Nosema* species, *Nosema ceranae,* which some suggest may be replacing the more familiar *Nosema apis*. *Nosema ceranae* is widespread throughout the UK, but *Nosema apis* still persists. Data from some EU countries suggests *N. ceranae* is capable of inflicting significant losses to commercial beekeeping operations. Other countries suggest *N. ceranae* to be a pathogen of minor significance. In the light of such conflicting information, we will summarise the current evidence on the distribution and impact of both *Nosema* species in the UK, including their association with certain honey bee viruses. Data will be presented from national surveys using species-specific real-time PCR protocols. In addition, data will be presented from a three year study of colonies containing a natural infection of both *Nosema* species at the National Bee Unit in York, England.

Effect of a diet, Nosema and virus infections on honeybee (*Apis mellifera carnica*) colonies

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In recent years, *Nosema* and virus infections have supposedly increased colony mortality worldwide. In order to contribute to the conservation of the indigenous population of Carniolan honeybee in Slovenia, we focused on the quality of the honeybee diet, winter survival and the influence of *Nosema* infections or viruses in colonies, giving special emphasis to the changing climate (e.g. the long, mostly rainless summer, mild winter, temperature oscillation in early spring). In late summer of 2009, when we observed a lack of nectar and pollen, the honeybee colonies received a different composition of diet substitutes (Apifonda®, pollen, vitamine Muvisel®; Apifonda®; no substitutes). At the same time, queens in the colonies were individually inoculated with spores of *Nosema apis* or *N. ceranae*. Samples of workers and feces of queens were collected monthly, four times in a row, in order to determine the presence of *N.apis/ceranae* and viruses. Colonies were equally prepared for the winter and fed with sugar syrup. During the experiment, the size of the brood and colony development were estimated; natural mite drop was counted as well. In the second part of our research, the newly-emerged workers were individually inoculated with a solution of different numbers of *N. ceranae* spores, and kept in cages. The inoculation was made in the spring and autumn season. Spores in cage-workers were frequently checked and quantified. Midguts were prepared for immunohistological analyses and the level of cell death was estimated. The longevity of infected cage-workers was also observed.

Negative effects of Nosema infection in honey production and vitality of honey bee colonies (*Apis mellifera iberiensis*) in Spain

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Nosemosis is one of the most frequently observed parasitic pathologies affecting adult honeybees, caused by *Nosema apis* and *Nosema ceranae*.
In this work, honey production and clinical evolution of 50 naturally infected colonies of *Apis mellifera iberiensis* during one year period is reported. In September 2007, all colonies were infected by *N. ceranae*, while only 26 of them were initially co-infected also by *N. apis* (this microsporidium was not detected during summer period; PCR tested). Colonies were randomly distributed into 5 groups of 10 colonies each: (C) control unmanaged colonies; (CS) control colonies fed with syrup only; (1T) treated with syrup+fumagillin in autumn; (2T) treated with syrup+fumagillin in autumn and spring; (4T) treated with syrup+fumagillin in autumn, winter, spring and summer.
After fumagillin treatment, the percentage of infected foragers decreased in all treated colonies, and in autumn, the percentage of *Nosema* infected bees was zero in the colonies of bees that consumed all the syrup with fumagillin. The group of colonies with 4 treatments per year were the most productive (24 kg. of honey/hive), but the group with 2 treatments/year got similar results in production (21 kg./hive) showing both groups a high vitality (number of adult bees/brood cells) compared with other groups. Control groups presented high levels of infected bees all year long, and were less productive (10 kg./colony) and less populated. The unmanaged control colonies registered the highest number of dead colonies, since the 40% of them were dead one year after. This work was supported by API-06-009.

Nosema ceranae as the agent of Type C Nosemosis in *Apis mellifera* Honeybees

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Data collected worldwide during the last years demonstrated that *Nosema ceranae* is an emerging pathogen of the 21th century. Current active scientific work is showing that this microsporidia presents differences in its genome, epidemiology and pathology when compared to *N. apis* Zander 1909, the traditional agent of Nosemosis till *N. ceranae* was found in European honeybees in 2005 (Higes et al., 2006; Huang et al., 2006). The disease caused by *N. ceranae* proposed to be called as nosemosis type C (COLOSS workshop, 2009) is characterized by the continuous death of highly infected bees, mostly foragers, having a clear effect on colony population and productivity, although differences in epidemiology has been reported throughout Europe. In addition, infected bees can be detected during all the year although no signs are easily seen in colonies in some moments of colony infection. Alterations reported at colony level (as honey harvest and colony development for example) and at individual bee level (immune-suppression, pathological lesions, host range, etc.) show us that *N. ceranae* should be considered as a major honey bee pathogen.

Effects of queen supersedure in Nosema spp. infected honey bees (*Apis mellifera iberiensis*) colonies

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Nosemosis is a common worldwide disease of adult honeybees caused by *Nosema apis* and *Nosema ceranae.* Nowadays, few alternatives are available to control the disease, because the use of fumagillin is restricted in many areas of the world. Since there is a direct repercussion of *Nosema* infection in individual worker bees and whole colonies, the role of queen in bee replacement is vital.A field assay was carried out to test the potential role of queen supersedure to avoid the negative impact of *Nosema* spp infection in honey bee colonies. Twenty colonies naturally infected with *N. ceranae*, (3 coinfected with *N. apis*) were monitored and randomly distributed into 4 groups of 5 colonies each: Group A were treated with fumagillin in autumn and spring; Group B treated with fumagillin in autumn and queen supersedure in spring; Group C fed with syrup in autumn and spring; Group D fed with syrup in autumn and queen supersedure in spring. Supersedure was enforced by removing the preexisting queen.
The results showed a significant decrease in the percentage of infected forager bees in colonies of group B, diminishing on average to a half lower parasitization after queen renewal. Similar effects were found in colonies of group A after fumagillin application, unlike the hives without treatment or queen supersedure, which showed high levels of parasitization (≥50%) all over the assay. In the case of house bees, infection disappeared after queen renewal and after fumagillin treatment also. Concerning honey production, group A was the most productive (26 kg./colony), followed by the group B (21 kg./colony). Groups C and D resulted less productive, with 15 kg./colony and 10 kg./colony respectively.
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Theme IV. BEEKEEPING AND BEE RESEARCH:
Symposium 13. Beekeeping and bee research in Turkey

Industrial apiculture in the Jordan valley during Biblical times with Anatolian honey bees

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Although texts and wall paintings suggest that bees were kept in the Ancient Near East for the production of precious wax and honey, archaeological evidence for beekeeping has never been found. The Biblical term “honey” commonly was interpreted as the sweet product of fruits, such as dates and figs. The recent discovery of unfired clay cylinders similar to traditional hives still used in the Near East at the site of Tel Re!hov in the Jordan valley in northern Israel suggests that a large-scale apiary was located inside the town, dating to the 10th–early 9th centuries B.C.E. This paper reports the discovery of remains of honeybee workers, drones, pupae, and larvae inside these hives. The exceptional preservation of these remains provides unequivocal identification of the clay cylinders as the most ancient beehives yet found. Morphometric analyses indicate that these bees differ from the local subspecies *Apis mellifera syriaca* and from all subspecies other than *A. m. anatoliaca*, which presently resides in parts of Turkey. This finding suggests either that the Western honeybee subspecies distribution has undergone rapid change during the last 3,000 years or that the ancient inhabitants of Tel Re!hov imported bees superior to the local bees in terms of their milder temper and improved honey yield.

A study on determining the effect of drug treatment season on Varroa (*Varroa destructor*) population growth

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Turkey has a big beekeeping potential with geographical structure, suitable ecology, rich flora and big colony numbers. Using wrong drugs at wrong time and insufficient struggle against the diseases and parasites, both reduce the productivity and affect the human healthy badly. *Varroa destructor*, which affect the colony productivity, is the most important parasites in the honeybee (*Apis mellifera* L.) colonies. Beekeepers use different chemicals for reducing or prevent the varroa destructor’ damages when they notice this parasite in their colonies.
This study was conducted to determine the effect of drug treatment season on *Varroa destructor* population in honeybee colonies. Same drug application were used at three different season (early Spring, Summer and late Autm) to test the effect of season. Ten honeybee colonies, which infestetion levels were close, were used for drug treatment and ten honeybee colonies were used for the control. Average effect of drug application of Early Spring(March), Summer(June) and Late Autm(October) were found as 69.72 %, 84.61 %, 80.22 % respectively. It was found an important stitasticall difference between the drug treatment season on effect of drug aplication on varroa population growt. Strugle of late Autm had 21 % more effectiveness than the strugle of Summer season. This study showed us that the strugle againist the varroa destructor should be done at late Autm or early Spring seasons.

Wing shape analysis of honey bee subspecies distributed in the Middle East using landmark method

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Honey bee subspecies in the Middle East include the subspecies *Apis mellifera anatolica, A. m. caucasica, A. m. cypria, A. m. meda, A. m. syriaca* and *A. m. armeniaca* discriminated by traditional morphometric and genetic analysis. Here, we used geometric morphometric approach based on landmarks in order to discriminate honey bee subspecies in the Middle East. A total of 702 colonies was evaluated from the distributional areas (Turkey, Cyprus, Iran, Iraq and Azerbaijan) in the Middle East. All colonies were described by means of 10 workers and 20 landmarks on the fore wings were identified. Landmarks were digitized by using tpsDIG to obtain 40 x, y cartesian coordinates. Landmark configurations were scaled, translated and rotated aganist the consensus configuration by GLS procrustes superimposition method. Aligned landmark coordinates were used as a data set for assigned subspecies groups and Multivariate analysis of variance, Pairwise tests were applied. Geometric morphometric data were also used as the data set for multivariate statistical analysis and UPGMA cluster analysis based on Mahalanobis distances was conducted. MANOVA revealed subspecies were significantly different for colony consensus average wing shapes ($P<0.001$). In the Canonical variate analysis, four main groups formed. Colonies of *A. m. cypria* and *A. m. meda* were clearly separated from other groups. However, the colonies from Thrace in Turkey and Crete have overlapping clusters which was supported by Pairwise test. Anatolian colonies and colonies of *A. m. caucasica* also formed an overlapping cluster as it is seen between Azerbaijan colonies and *A. m. meda*.

How changing population dynamics affect honey bee colony performance

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Division of labor and population dynamics are important factors for ecological success and performance of honey bee colonies. We examined effects of colony population dynamics on colony performance by manipulating brood age structure in different colonies. We hypothesized that change in brood age structure will result in change in colony age structure and consequently in colony foraging performance as measured by pollen and honey stores. We prepared three different groups: “Older worker biased”, “Younger worker biased” and “control” colonies. The age structure hypothesis predicts that older worker colonies with larger group of only older brood will have more foragers than control and younger worker colonies during the nectar flow period, resulting in higher honey and pollen stores. Alternately, as predicted by plasticity hypothesis, younger and older worker colonies may resemble control colonies in honey and pollen stores. Our result showed that honey stores were higher in older worker colonies than control and younger worker colonies, as predicted by the age structure hypothesis. Although the trend was in predicted direction, we found no statistically significant difference between control and younger worker colonies for honey. However, different to predictions of both plasticity and age structure hypotheses, pollen storage area was larger in younger worker colonies than control colonies. In addition, older worker colonies did not have significantly more pollen storage area than control and younger worker colonies. We also discuss how these manipulation techniques could be useful in practice for honey production during early honey flows or in preparing colonies for pollination services.

Genetic diversity of honey bee ecotypes in Turkey based on microsatellites

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In the present study Genetic analyses of diversity were performed on five honey bee population. Microsatellites which are among the preferred molecular markers at subspecies level because of their high polymorphism and codominant inheritance were used to study genetic differentiation among five honey bee population. In total 86 honey bee colonies were genotyped for 8 microsatellite markers (A008, A024, A043, A088, A113, Ac306, Ap068, Ap226, Ac306).We dedected heterozygosity levels (He), mean number of alleles per population, presence of diagnostic alleles and pairwise Nei values. High percentage of genetic variance was found within populations (94%). Mean heterozygosity (He) was to change between 0.484-0.551. It was determined a very high level of genetic divergence between Yığılca province of Düzce city and Kırklareli in Thrace region based on pairwaise population Nei genetic distance (0.295).This study provides with us a large amount of information which can be useful to develop genetic conservation strategies.

Research of honey bee colony losses and deaths in Marmara region

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Within the boundaries of the nine provinces of the Marmara Region which covers his work on investigation of bee deaths and losses as a result of the data obtained in the form of an evaluation report was prepared. This study of death and loss of greater than two months after the news came on the 2 conducted research and field work. In the first study in 9 provinces of Marmara Region Beekeepers Bee Breeders' Association and has held meetings with, beekeepers bee talks about death and loss have been made. 2. Research from the governor of Edirne and Tekirdag and losses reported on the death of bees in July 2007 on the analysis carried out in the region.Pendik VKAE'de of purity of the samples were analyzed. With data obtained from research and survey information from the assessments made as a result of the report prepared and presented to the Ministry with proposals for solutions. 1.ve 2 The bee death and loss of field research in order to be around 20% and 80% were determined. Highlights the issues, sunflower fields of death and loss of locally are great. Sunflower seeds that were coated with the imidocloprid.

Nosema cerenae and Nosema apis in colony collapsed apiaries of Hatay

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Hatay region has been serve as an useful wintering region of Turkey because of it's mild winter climate and early starting spring. Wintering region is bordered with Mediterranean Sea among the west border. East side of the wintering region is bordered with Syrian mountains from the behind. So the humid etesian winds blows pass through the wintering region. We detected *Nosema cerenae* with mixed infections in most of the collapsed colonies Molecular differentiation of Nosema species were performed with PCR. Occurrence of *N.cerenae* was found commen more than *N.apis* in collapsed apiaries of Hatay wintering region. Preventive cautions against to nosemosis may achieved by sustainable and widespread veterinarian services with awareness raising of beekeepers.

Migratory Beekeeping in Turkey and Related Problems

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Turkey is one of the important producers of honey in the world with approximately 50.000 tons of annual production and 4.000.000 beehives. Turkey is a rare country where one experiences four seasons at the same time which results in a wide range of flora and long honey season. There are 40.000 beekeeper families in Turkey most of whom are migratory beekeepers. Production of honey is generally the only source of income for migratory beekeepers who prefer to carry their hives from one region to another during the main season to increase their honey yield. In this study, results of a survey with participation of 2255 beekeepers are given and the problems about migratory beekeeping in Turkey are discussed within the framework of this data. Results showed that, 45,4% of beekeepers in Turkey are migratory beekeepers, whose 62,7% has an experience equal or more than 15 years and 65,3% has 100 or more beehives. 32,3% of migratory beekeepers declared that they are using veterinary drugs against bee diseases. Main problems are determined as; insufficient places for migratory beekeepers to stay overnight, easy spreading of bee diseases especially varroa and European fool brood, genetically contamination between bee races, transportation and insufficient places for settlement of bee hives. Details of these problems and corresponding solutions are explained in the study with an aspect from legal regulations, need of modernization, importance of training and raising awareness in the society about the contribution of beekeeping for agricultural production.

Theme III. BEE BIOLOGY and ECOLOGY:
Symposium 9. Developmental and behavioral plasticity in eusocial bees

Behavioural plasticity underlies the solitary-eusocial transition in the socially polymorphic sweat bee *Halictus rubicundus*

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Eusociality has evolved just a few times in the bees. Interestingly, some sweat bee (Halictidae) species straddle this major evolutionary transition; they are socially polymorphic, exhibiting both solitary and social behaviour. Previous studies of socially polymorphic sweat bees have suggested a genetic basis to the social transition. We have tested whether there is a genetic underpinning of this transition in the socially polymorphic sweat bee *Halictus rubicundus*, which exhibits solitary behaviour in the cooler north of its European range and sociality in the warmer south. Our population genetic data reveal subtle genetic differentiation between populations, though the level of differentiation between social and solitary populations is no greater than expected by geography alone (isolation by distance). These data suggest little or no reproductive isolation between solitary and social populations of *H. rubicundus*. Our common garden experiments, in which we cross-foster social *H. rubicundus* into a solitary environment and *vice versa*, indicate plasticity in social behaviour. If there is a genetic underpinning to the social transition, our results suggest that it has a simple genetic architecture, possibly controlled by one or a few key, regulatory genes.

Functional genomic approaches to unravel the physiological basis of honey bee caste development

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While gene expression analyses were cumbersome before 2006, the availability of an annotated genome and powerful genomic tools turned gene expression assays into straightforward approaches, benefitting our understanding of caste development. The insulin/insulin-like signaling (IIS) pathway in conjunction with the target-of-rapamycin (TOR) pathway have received most attention, showing that TOR function is required for queen development. The results for the IIS pathway were, however, counterintuitive, as insulin receptor expression is down-regulated in queen larvae during the phase of most accelerated growth. So as to understand this paradox we currently focus on hypoxia signaling as a possible mediator of IIS function, since oxidative metabolism has long been known to play a role, even though little understood, in honey bee caste development. Expression analyses now showed that core genes of hypoxia signaling are over-expressed in worker larvae. In parallel with such candidate pathway analyses we are investigating how gene networks are organized in caste-specific organ development. By suppression subtractive hybridization (SSH) analyses we identified sets of genes that are differentially expressed during development of the larval ovary and leg imaginal discs. A surprisingly large portion of the SSH ESTs represents genes of unknown function, micro RNAs, and even genes that have not yet been predicted in the honey bee genome. Investigating the expression and function of these genes should render novel insights on how caste phenotypes are built from networks integrating conserved signaling pathways with novel specific function genes.
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Flexibility of division of labor in honey bees: all explained by a simple model

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The temporal polyethism of worker bees is highly flexible. The normal behavioral development, from nurse to foragers, can be accelerated, delayed or even reversed depending upon the colony conditions. For example, if a colony is made entirely of newly emerged bees, some workers would become foragers around age of 7-10 days, instead of at the normal age of 14-30 days. Conversely, if a colony is made entirely of foragers, some foragers would “revert” to become nurses and take care of the brood and queen instead of continue to forage for food. Plasticity in behavioral development also appears to be mediated by juvenile hormone (JH). Precocious foragers have a precociously high JH titer, overage nurses have a low titer, and bees that revert from forager to nurse show a drop in JH titer. These results suggest that changes in colony conditions act on the endocrine system to cause changes in temporal polyethism.

We were interested in the mechanism of how workers “transduce” the colony condition into appropriate changes in physiology that ultimately mediates the changes in behavior. There are four ways workers could obtain information about their colony status. One is centralized control in which one or more workers monitor the colony status and direct other members to specific tasks. While in small colonies it is possible for the queen to do so, there is no evidence for centralized control in honey bees. Another way is for each and every worker to gauge the colony needs and respond accordingly. This is also unlikely because of the large population size (15,000 - 50,000 members per colony), and the limited neural capacity of worker brains (1 million neurons). Alternatively, workers could obtain colony information vial local cues that is correlated with the global colony status, analogous to what cells do in a metazoan organism. Cell development is mediated by interactions either with other cells or with the extracellular matrix. Workers could obtain local information via two similar pathways. In the "worker-worker" pathway the colony information is acquired indirectly while adult workers interact with each other, perhaps via trophallaxis. In the "worker-nest" pathway this information is acquired while interacting directly with the nest and its contents, such as the colony's food stores and brood perhaps during bouts of "patrolling" behavior.

Two pieces of evidence suggest that workers assess colony conditions mainly through interaction with other workers. When individual workers were reared in isolation away from the colony, for 1 week, their rates of juvenile hormone synthesis elevated prematurely to the levels that were usually seen in normally aged foragers (3-4 weeks old). These workers foraged significantly earlier than bees that were reared in a colony. We suspected that the isolated workers developed prematurely because of a lack of inhibition from old bees. Further experiments using single-cohort colonies showed that, indeed, the developmental fate of young workers is determined by the presence or absence of foragers in the colony. When foragers or soldiers were "transplanted" into a single cohort colony, the precocious development was inhibited in the resident bees, apparently due to the inhibition from the transplanted old bees. Resident bees still developed prematurely in the control colony, where only young foreign bees were transplanted. Closing the colony entrance, therefore eliminating any chance of nest modification by transplanted foragers, did not reduce the inhibition of foragers upon the resident bees. This suggested that young bees did not base their developmental decisions upon changes of nest environment, because if that is true, the colony with foragers transplanted but entrance closed should behave as though no foragers were present, i.e. show accelerated development.

Based on these results, we developed a social inhibition model that explains all three forms of behavioral development.

Proteotyping division of labor in worker honeybees

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Honeybee workers are essentially sterile female helpers that make up the majority of individuals in a colony. They display a marked and usually age-related division of labor: young workers perform in-nest tasks such as nursing the brood
and cleaning the hive, while older workers forage for pollen and nectar in the field. Recent technological advances have made it possible to unravel changes on the transcriptome, proteome, and metabolome level that are associated with this behavioral divide. Out of these three levels of physiological organization, the proteome can arguably be seen as the centerpiece that facilitates changes on all other levels.I will report on studies that investigate proteomic changes associated with the onset of foraging from different perspectives. First, I will present data that shows general differences in the proteomes of nest and forager bees. Second,I will show how behavioral and age-related proteomic changes can be decoupled and analyzed separately using colony-level manipulation. Third, I will report on a study that monitored proteomic changes preceding the onset of foraging in the honeybee fat body.Overall, these studies make use of different genotypes, RNA interference technology, and colony manipulation to provide evidence for a major behavior-associated remodeling of protein networks on the whole-body and tissue-specific level in worker honeybees.

Effects of carbohydrates on the development and sugar responsiveness of honey bees (*Apis mellifera L.) reared in vitro*

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Honey bees (*Apis mellifera* L.) are used as a model organism for evolution, developmental biology, nutrition, aging, social behavior, genomics and epigenetic. Younger larvae are fed progressively by the nurse bees with a mixture of mandibular and hypo-pharyngeal gland secretions. Older larvae are fed honey and pollen. We have studied the effects of fructose and glucose concentrations on the development of honey bees reared *in vitro* and the response of the adults to the dietary sugars. Honey bee larvae require carbohydrates for development, synthesis of hormones and metamorphosis. They can utilize both fructose and glucose. The amount of sugar in diets and the sugar composition affects the larval weights, adult live weights, ovariole numbers and sugar responsiveness.Worker larvae were fed with diets containing 12% glucose, 12% fructose or 6% glucose + 6% fructose. After emergence, adults were maintained on a diet consisting of the same sugars for 48 hrs. Bees were then harnessed and their response thresholds for fructose, glucose or mixture of glucose and fructose were determined. Bees showed a lower response threshold for the sugar on which they had been raised. Bees raised on the diet with the equal mix of sugars showed equal responsiveness to each sugar. These results suggest that diet during rearing can affect sensitivity to specific sugars.

The putative role of male sex pheromones in bumblebee cuckoohost interactions

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In social insects, the high energetic costs of brood care have promoted the evolution of cheaters that exploit workers services of conspecifics or heterospecifics. In Bumblebees, all the species of the subgenus *Psithyrus* have lost their worker caste and are completely dependent on hosts to produce their sexuals. One of the most striking challenges for these social parasites is how to escape from the detection and rejection by their hosts. Many studies have shown how the *Psithyrus* queens overcome host recognition systems and successfully enter host colonies. However, once a social parasite has successfully usurped a host nest, its emerging offspring still face the same challenge of avoiding host recognition. How cuckoos offspring fool their hosts has been highly investigated in birds but poorly studied in social insects. We assume that cuckoo bumblebee females might camouflage themselves by decreasing pheromonal glands production and acquiring host nest odor. However young males already produce high amounts of species specific cephalic labial glands secretions. Host workers might be able to recognize them. Therefore, *Psithyrus* males might use another strategy to escape from workers detection, by (i) producing facilitating signals that enables them to be accepted by the host workers or by (ii) producing a chemical blend that does not appear as allospecific to the hosts.
The aim of this study is to look at how males of the cuckoo bumblebee *Bombus vestalis* fool *Bombus terrestris* workers during their intranidal life, using chemical analyses of their cephalic labial glands secretions and behavioral recognition assays.

Larval and nurse worker control of developmental plasticity in honey bee queens and workers

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Polyphenism, the expression of alternative phenotypes in response to environmental cues, is widespread and is thought to evolve via the modification of developmental plasticity. Queen-worker dimorphism in insect societies is an exemplar polyphenism that forms the basis of the reproductive division of labor characterizing eusociality. Social evolution in honey bees has produced strong queen-worker dimorphism for traits such as ovary size and body size that are sensitive to larval nutrition. Nurse workers strictly control larval nutrition and thereby regulate larval developmental trajectories and the resulting expression of queen or worker phenotypes. As a result, the honey bee developmental program includes larval components that determine plastic growth responses to the nutritional environment and nurse components that regulate the nutritional environment. We studied the contribution of these two components to variation within and between two pairs of honey bee lineages for body size and ovary size in queens and workers. The lineages differed in the degree of social control of development for body size, ovary size, and the body-ovary size relationship, and the degree of developmental plasticity for these traits in response to variation in the nutritional environment. Based on patterns in these differences, we suggest that larval and nurse genetic components were available for natural selection to act on during the evolution of honey bee queen-worker dimorphism, affecting the developmental plasticity of body size and ovary size, and allometric scaling relationships between these traits.

Socially-mediated plasticity in circadian rhythms and the molecular clockwork

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Inhibition of JH signaling is important for the regulation of worker behavior and physiology and plays a key role in social organization, but JH influence on worker social behavior appear to vary between bee species. In honey bees, inhibition of JH levels slows down behavioral maturation that is associated with worker division of labor. In bumble bees, the presence of the queen inhibits worker reproduction via decreased juvenile hormone levels, but it thought to have no effect on division of labor. JH has pleiotropic effects and the pathways downstream of JH are predicted to at least partially diverge in order to accommodate the differential influences on division of labor and reproduction in adult workers of the two species. We found that allatectomized *Bombus terrestris* workers do not develop their ovaries even under queenless conditions that typically promote worker reproduction, confirming the gonadotrophic function of JH. In honey bees we characterized JH binding proteins. We studied in detail the expression of one of these proteins and found it to be regulated by JH. We further examined in bumble bees the expression patterns of a transcription factor, *Krüppel-homolog 1* (*Kr-h1*). *Kr-h1* expression is downregulated in honey bee workers upon exposure to queen pheromone, and upregulated in foragers. In the bumble bee, *Kr-h1* levels were similarly downregulated by the presence of the queen or dominant worker. *Kr-h1* levels were also associated with JH-mediated changes in reproductive state, but elevated levels were not associated with foraging behavior. These results suggest that *Kr-h1* is a part of a JH signaling pathway that in social bees is linked to social regulation, but was differentially co-opted during social evolution of bumble bees and honey bees.

Functional mapping of the circadian network and its role on the sleep-like state

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Circadian rhythms are biological oscillations with a period of approximately 24 hours that serve as endogenous timers to synchronize internal physiological processes such as sleep with environmental conditions. Although there are many cells throughout the mammalian and insect body that express the essential molecular components of the circadian system, it is believed that only a few cells in the brain are the “Masters” controlling the circadian program and that the other “slave” cells just obey instructions. In honey bees there are no transgenic methods to examine the network functionally. We are using the *Drosophila* model instead. Any hypotheses elucidated can also be tested in the honey bee model with pharmacological or behavioral interventions in the honey bee. Here we show that the evening locomotor activity of *Drosophila melanogaster* can occur in the absence of the “Master” cells (Ventrolateral Neurons or LNvs) and is abolished by the elimination of the Dorsolateral Neurons (LNds). Conversely, the morning locomotor activity can occur without the LNds but is abolished by the genetic ablation of the LNvs. Additionally, we show that the LNvs express the $GABA_A$ receptor subunit (RDL) and that these neurons are inhibited by GABAergic input during sleep. Taken together, our findings indicate that behavioral rhythms are controlled by multiple oscillators that work in synchrony to modulate different aspects of circadian behavior. We propose that the central pacemaker neurons (LNvs and LNds) promote wakefulness and have mutual inhibitory connections with a group of GABAergic neurons that promote sleep by inhibiting the circadian pacemaker neurons.

Theme I. BEE LOSSES: Symposium 3. Monitoring, Diagnostics, and Pathogens
Organizer: Romée van der Zee

Bee Mortality and Bee Surveillance in Europe

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This project, founded by the European Food Safety Agency, sought information on both the prevalence of honey bee colony losses, and the surveillance systems found in 27 European countries. Data were obtained from 24 countries. Each of the surveillance systems collecting these data was evaluated. In addition, a thorough literature search of the existing databases was completed.

The main conclusions from project activities can be summarized as follows:

- General weakness of most of the surveillance systems;
- Lack of representative data at country level and comparable data at EU level for colony losses;
- General lack of standardisation and harmonisation at EU level;
- Common consensus of the scientific community about the multifactorial origin of colony losses in Europe and in the United States and insufficient knowledge of causative and risk factors for colony losses.

The project makes recommendations in the following area:

- Implementation of a sustainable European network for coordination and follow-up of surveillance on colony losses;
- Strengthen standardization at European level by harmonization of surveillance systems, data collected and by developing common performance indicators;
- Build on the examples of best practice found in existing surveillance systems for communicable and notifiable diseases;
- Undertake specific studies that build on the existing work in progress to improve the knowledge and understanding of factors that affect bee health (for example stress caused by pathogens, pesticides and their interactions) using appropriate epidemiological studies

The set up of the sustainable coordination team at European level to enable effective surveillance program activities at the European level

Honeybee Colony Losses 2009-10 in 23 countries using mainly the COLOSS 2009-10 questionnaire

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Within the Workgroup Monitoring and Diagnosis of the COLOSS network a questionnaire was developed to collect mortality data 2009-10 internationally. First results of the countries who adapted to this questionnaire will be presented. Conclusions will focus on Colony Losses in relation to CDS (Colony Depopulation Syndrome), Bee-Race, Pollination, Migration and Economic Value.

Quite some surveys suffered coverage problems. The questionnaire questions were sometimes changed by surveyors in a way that changed the outcome in essence. Some countries presented different surveys, for various reasons, which resulted in difficulties to interpret contrasting information.

The main drawback was that the questionnaire was not suitable to collect loss data from operations where colonies were actively managed within the timeframe of the study.

A spatial analysis of the data is in progress.

Detection of the major honeybee pathogens by Multiplex PCR: from honeybees to other organisms

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Over the past few years, weakening, collapse and mortality of honeybee colonies have been reported in many countries. The lack of identification of precursory signs and characteristic symptoms leads to confusion and misunderstanding concerning these recurring cases. A variety of fungal, bacterial and viral infections are commonly observed but these pathogens are not routinely identified. This leads to uncertainty about the causes of this phenomenon. The aim of this study was to develop molecular tools to detect rapidly twelve major microscopic pathogens of the honey bee. Three multiplex PCR have been developed to discriminate the presence of three fungi (*Ascosphaera apis*, *Nosema apis* and *Nosema ceranae*), two bacteria (*Paenibacillus larvae* and *Melissococcus plutonius*) and seven viruses (Acute bee paralysis virus ABPV, Black queen cell virus BQCV, Chronic bee paralysis virus CBPV, Deformed wing virus DWV, Israeli acute paralysis virus IAPV, and Sacbrood virus SBV). These molecular tools have been validated for the detection of these twelve pathogens in adult and brood honeybee samples collected in France. In addition, organisms closely related to honeybees such as wasps and ants, or macro-pathogens of honeybees as *Varroa destructor* were also analyzed with the three Multiplex PCR. Results have shown than these organisms carried at least one honeybee virus. Perspectives open by these molecular tools are exposed and discussed.

Detection of pathogens In honeybee mortalities observed in France during 2007 to 2009

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Significant mortality rates of adult honeybees were observed during the 2007, 2008 and 2009 beekeeping season in France. These outbreaks of bee mortalities have led our laboratory to conduct studies in order to determine the pathogens involved in this phenomenon. Bee samples from apiaries located in various parts of France were sent to our laboratory for diagnosis by beekeepers and veterinary services. Two sets of samples coming from apiaries presenting or not troubles (e.g.: bee mortalities, abnormal symptoms as trembling or crawling bees, black bees, low activity) were analyzed.Our laboratory carried out the detection of the major honeybee pathogens: three fungi (*Ascosphaera apis*, *Nosema apis* and *Nosema ceranae*), two bacteria (*Paenibacillus larvae* and *Melissococcus plutonius*) and seven viruses (Acute bee paralysis virus ABPV, Black queen cell virus BQCV, Chronic bee paralysis virus CBPV, Deformed wing virus DWV, Israeli acute paralysis virus IAPV, and Sacbrood virus SBV) by conventional PCR and/or real-time PCR.
Detection of pathogens was assessed for correlation with the troubles described in the purpose of determining the implication of these various pathogens. Some surveyed apiaries presented high viral loads confirming the diagnosis of the chronic paralysis and highlighting the role of CBPV in bee mortalities

Can we think differently talking about European foulbrood causalities?

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During the control program for early detection American foulbrood from brood nest honey in Bosnia and Herzegovina we find lots of bacterial contamination that we usually classified as secondary infections in EFB disease or intestinal microbiota of the honey bees.
The theory and rational thinking sometimes are not in a harmony. So how can we harmonize our method of questioning with natural truth? All is challenge for better understanding disease complex that we today frequently declare as EFB. Previously, disease has been attributed to *Streptococcus api*s, *Bacillus alvei* and *Bacterium eurydice* . Today it is accepted that EFB is caused by *Melissococcus pluton*, which is a gram-positive bacterium. The other bacteria previously thought to cause EFB are stated as commonly associated secondary bacteria. That status of secondary bacteria in honey bee environment and honey bee product is something that can be evaluated correctly. How can we call simply EFB, disease when we have different clinical pictures and with totally different bacterial load on that particular clinical substrate. Additionally, disease nomenclature in the different countries see EFB differently: for example in the Russian beekeeping literature there are two separate disease entity that in OIE standards are just described as one disease, EFB.
In the veterinary medicine field we do not have many disease with one name for two or tree bacteriological different causes. It will be totally unfair to see secondary infections with *Bacillus species* as a coincidental biota or secondary infections as they have naturally different microbiological and possibly clinical patterns.
Maybe we can see two different disease concerning clinical pictures that we today see as simply as EFB. It is difficult to believe that one sporogen bacterium as *Paenibacillus alvei* is possible easy to control as *Melissococcus plutonius*. That can be reason that actual control strategies today in the EU are not efficient enough. After a big avalanches of sophisticated diagnostic tests in past decade concerning EFB (*Melissococcus pluton)* beekeeping practice is still with no standard and accepted proper protocol for EFB control.
In presentation we will discuss more about results and statements.

Why do bees survive well in Northern countries in spite of unkind environment?"

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There are 250 000 honey bee colonies in Finland, Sweden, Norway and Denmark in cold climate areas. In all of these Nordic countries *Varroa* infestation has spread out during the last two decades and there are only few areas with small number of bee colonies where *Varroa* has not been detected. Also the *Varroa* associated viruses and both the the *Nosema apis* and *Nosema ceranae* have been detected in many areas. *Acarapis woodi*, *Paenipacillus larvae*, *Melissococcus, Ascosphaera apis* have found to be common in several areas, where the colony density is comparative high. The circumstances for bees should be very rough, when the coldness and flightless season may last even seven months. It could be expected that these factors cause heavy winter losses.
In spite of all these negative environmental factors, average bee losses have not been shown to be more than 10 - 15 % during a long period surveys. We classified the winter losses in two categories: the "common problems" and "unforeseen reasons" to find out the uprising new factors and changes in environmental or human factors affecting
honey bee over wintering. The new factors are easily covered by bias caused by variation of common annual winter losses. The cyclic changes and the correlation of weather conditions and the influence of parasite pressure to winter losses will be presented.

Estimation of honeybee colony losses within professionnal beekeepers in France during winter 2008/2009

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During last years, professional french beekeepers have found an increase in winter honeybee colony losses (mortality, weakness, diseased or queennless). A lot of causes have been mentioned to try to understand those losses, like phytosanitary treatments, parasites, diseases, lake of biodiversity in farmlands, but it have been impossible to clearly conclude.
In 2007, the French technical and scientist institute for beekeeping and pollination launched a survey in aim to improve general knowledge about winter bee losses. The questionnaire contains questions about beekeeping practices for wintering preparation, colony background during the season, environment of apiary.
The first year was a pre-study, 168 professional french beekeepers (more than 150 hives) were randomly selected out of 782 beekeeping farms. The losses rate was 29% (IC95% = [26% - 32%]) at the national level and following results have been obtained with this first survey:
In 2008, a second sample group has been defined and the questionnaire has been filled. Preliminary results for possible causes show a correlation between availability of food, strength of the colonies and varroa pressure with the losses. During winter 2008/2009, the losses rate was 23% (IC95% = [21% - 25%]).
A deep study of those data has been done, in the aim of confirm the typology, the risk factors and their interactions, and to develop prediction models of the loss rates.
We intend to extend this national survey over several years to get a close monitoring of loss rate. We'll be able, next September 2010, to show the first figures on bee colony losses during winter 2009/2010.

First detection of honey bee pathogens in nests of the oriental hornet (Vespa velutina collected in France)

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The Asian hornet *Vespa velutina* L., originally from tropical regions of Asia, was accidentally introduced into the South West region of France. This hornet has adapted well to its new environment colonising various areas. *V. velutina* is one of the most adapted hornets at catching honeybees on the wing, thus becoming a major threat for apicultural industry. If a honey bee colony becomes sufficiently deprived of workers, *V. velutina* will enter the hive and feed on the honey and remove the brood. *V. velutina* nests are exposed to honey bee pathogens through feeding on *A. mellifera* workers. We studied the presence of 9 honey bee pathogens in *V. velutina* nests and the presence of replicative RNA forms of 2 viruses (BQCV and SBV). Our results convincingly demonstrate the detection of SBV, BQCV and DWV in *V. velutina* adults, larvae and nymphs. CBPV and ABPV were also detected but to a lesser extent. It was found that at least two of the honey bee viruses (BQCV and SBV) were able to multiplicate in *V. velutina* (RNA minus strand detected). The detection of replicative form did not correlate with diseased-like aspect of the larvae. Our results show that honey bee viruses could get through heterospecific barriers which might lead to new strategies to control the Asian hornet populations.

Prevalence of viruses and Nosema spores in reared queens, *Apis mellifera carnica* (Pollmann, 1879)

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Honeybee queens were collected from queen breeding stations across Slovenia during the rearing seasons 2006 and 2008. In both years, 612 queens from 27 apiaries were sampled. Queens were weighed, dissected and prepared for morphological measurements (wings, head, number of spermatoza and ovariols) and pathogen analyses (*Nosema* spores and viruses). The attendants were also checked for spores, and *Nosema* species was determined by PCR. In 2006, queens were ABPV, DWV and SBV positive in 12.2, 8.7 and 1.2 %, respectively. BQCV was not detected. In 2008, DWV was most frequently present in queens and attendants in 65.3 and 36.1%, respectively, and BQCV in 23.6 and 13.9 %. ABPV was the least present (4.2 %), and only queens were SBV positive (8.3 %). Attendants and queens reared in 2008 were *Nosema* positive in 76.7 and 5.6 %, respectively; results of molecular tests found *N. ceranae* in all positive worker and queen samples. In 2008, the average number of spores in queens and attendants was 0.68 and 8.66 million, respectively. The highest number of spores amounted to 27.43 million and the minimum number amounted to 1.29 million. Queens from 2006 were all negative (no spores were found). The results of morphological measurements were compared and evaluated.

Theme IV. BEEKEEPING AND BEE RESEARCH:
Symposium 12. Bee Products

The estimation of unifloral Acacia honeys harvested in Poland on the basis of their pollen analysis and fructose/glucose ratio

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Samples of Acacia honeys, in number of 30, that were determined by beekeepers as unifloral were examined in 2008. The qualitative and quantitative pollen analyses and glucose/fructose ratio as the characteristic feature for this honey were examined. According to the Polish Standards the unifloral Acacia honey should contain over 30% of *Robinia* pollen. The total number of pollen grains in Acacia honey, according to the Maurizio classification, should be lower than 20 000 pollen grains per 10g of honey. According to the literature fructose/glucose ratio for Acacia honeys should be higher than 1.4. Altogether 15 samples from 30 examined honeys contained over 30% of *Robinia* pollen. Also in 15 samples the total number of pollen grains was lower than 20 000 per 10g of honey. However not in every case the content of the *Robinia* pollen over 30% was in interrelation to the total number pollen grains lower than 20 000 per 10g of honey. Instead the fructose/glucose ratio was significantly lower than 1.4 only in one sample (F/G = 1.2). Differences between results of qualitative and quantitative pollen analyses in the relation to the fructose/glucose ratio can be due to the additional pollen coming from bee bread.

Determination of origin in honey by aroma profile analysis

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Honey production and trade is one of the important sources of finance in the world. Prices are strongly related to the quality thus floral and geographical origin of honey which is an important grading factor for the international honey market. For mono floral honey types, it is possible to determine the floral origin of honey by certain techniques like determination of the dominant pollen. But for multi floral honey types, such techniques are not always enough because of the fact that bees may take only pollen from some flowers and may take only nectar from others. Other techniques are needed in that case both for confirmation and determination of origin in honey.
In this study, aroma compounds of a total of 150 honey samples from Turkey were analyzed by a purge and trap, gas chromatography mass spectrometer system and the results were compared as an indicator of their geographical and botanical origin. For this purpose, 15 pine honey samples from 6 different regions of Turkey (Bergama, Marmaris, Köyceğiz, Söke, Muğla, Isparta), 63 polyfloral honey samples from 7 different regions (Yüksekova, Şemdinli, Muş, Kayseri, Bingöl, Elazığ-Hazar and Van) and 72 monofloral honey samples (Urfa region cotton, Aegean region cotton, chestnut, heather, sunflower, canola, citrus and eucalyptus) were analyzed. A total of 150 aroma compounds were isolated from honey samples. Each sample showed a different aroma profile, with some compounds missing in some samples. 11 compounds were found to be present in 67% of all pine honey samples and not found in polyfloral honey samples. These compounds were 2-methyl-furan, 3-methyl-2-butanone, 2,5-dimethyl-furan, alpha-pinene,beta-pinene, 1-methyl-3-(1-methylethenyl)-cyclohexene, trans-caryophyllene, 2-cyclopropyl-pentane,a monoterpene and two unknown. 6 compounds were found to be present in 70% of all polyfloral honey samples and not found in pine honey samples. These compounds were, 2-methyl butanenitrile,2-methyl 2-butenal, herboxide second isomer, 1-octen-3-ol, alpha-terpinolene, nonanenitrile. Also some compounds were considered as good markers for mono floral honey types like propanal, 2-methyl- and 2,6-dimethyl-1,3,5,7-octatetraene, E which were specific to only heather honeys. The Principal Component Analysis (PCA) showed the most important compounds for each honey collected from different regions.It was concluded that aroma profiling is a useful, promising tool for determination of geographical and botanical origin of honey which may be used in combination with other techniques like pollen analysis.

Flavours of heather (*Ericaceae*) honeys: organoleptic and instrumental analyses

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Honey flavours are major parameters in determining both the quality and origin of this foodstuff. A number of studies on sensory and instrumental procedures to describe the variety of honey flavours have been reported. With regard to sensory analyses, the International Honey Commission proposed a harmonized terminology, and a routine method for honey quality evaluation. In respect of instrumental methods, the vast majority of procedures are based on gas-chromatography-mass spectrometry (GC-MS) determinations, after extracting honey volatile and semivolatile compounds by several methods. As heather (Ericaceae) honeys are very appreciated by their strong flavour, we have carried out both sensory and GC-MS analyses on 20 samples of honeys with Ericaceae nectars, most of them Ericaceae unifloral honeys. We have researched the volatile and semivolatile compounds that have been responsible for the aroma described after an olfactory and tasting assessment using a panel of five assessors who employed a sensory analysis defined protocol. The aroma described with sensory analysis has been floral, with bitter and woody accents, and a fresh aftertaste. Among the compounds analyzed by GC-MS after extracting volatile and semivolatile compounds employing ethyl acetate, the most relevant ones have been the aliphatic isomers *levo*- and *meso*-butane-2,3-diol, the monoterpene hotrienol, and the benzene derivative phenyllactic acid.

Non-aromatic organic acids of honey: significance and analysis

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Non-aromatic organic acids are minor constituents of honey. Nevertheless, they play an important role as factors for the characterization of both botanical and geographical origins of this foodstuff. Some organic acids are used as treatment against *Varroa* infestation. High levels of acetic acid can reveal honey fermentation. Furthermore, non-aromatic organic acids are related to antibacterial and antioxidant activities of honey. Several methods have been applied to determine honeys' non-aromatic organic acids. We have compared the precision, recovery, sensitivity, speed and cost of enzymatic, high performance liquid chromatography (HPLC), as well as capillary zone electrophoresis (CZE) procedures to analyze non-aromatic organic acids of honeys from several botanical sources (multifloral, eucalyptus, chestnut and clover). We have found several advantages and disadvantages of each of them, concluding that enzymatic methods are very specific, precise and accurate, but they are tedious and time consuming. HPLC procedures show good sensitivity, versatility and reproducibility, but interferences must be removed, so they are also time consuming. CZE determinations are very simple, rapid and low cost, but they are less sensitive and more difficult to reproduce. Thereby, the method of choice depends on the acids that are going to be analyzed.

Efforts to explain the great variability of diastase activity in monofloral honeys

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The use of Diastase activity has been criticized very strongly because of its extreme variability the pass 50 years, but no research was attempted to explain this variability.
In this research samples of honey were collected from bee colonies that had received the same manipulation, were in the same area and foraged the same crop at the same time. The diastase activity, electrical conductivity, water content, HMF, sugars and pollen spectrum were analyzed. In addition feeding tests with different concentrations of syrup and thymol were made.
The honey that had been collected was characterized by great variability of diastase. The variability of the other physicochemical characteristics ranged in logical levels. Syrup honey that had been produced from feeding test had low diastase without much variability. When thymol was incorporated in that syrup, its consumption was slower. The slower the bees get the syrup the more diastase they put. This explain why the intense flow of nectar, usually yields low values of diastase activity.
We further examined the effect of the number of bees that exist in one colony on the concentration of diastase of the honey they produced. Strong colonies collect and elaborate faster the nectar than weak colonies and they put different concentrations of diastase.

Migration of residues of chloramphenicol from contaminated beeswax foundations to honey

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In Europe chloramphenicol is not allowed to be administered to food producing animals. Nevertheless in China chloramphenicol was used in beekeeping, causing problems with the presence of residues in honey. This resulted in an European temporary ban on the import of Chinese honey. Due to the shortage in Europe of local beeswax for the production of wax foundations, beeswax is imported from other parts of the world, including China. A method for the detection of residues of chloramphenicol was developed and a contamination in a commercial wax foundation sample was found.
A migration test was set up to study if chloramphenicol-containing beeswax could lead to contamination of honey. Blank beeswax, free from chloramphenicol, was spiked with chloramphenicol at three different levels (20, 200 and 2000 µg/kg) and used for the fabrication of wax foundations. After framing, the wax foundations were placed in different hives to be further constructed by the honeybees to honeycombs and filled with honey. Once the cells sealed by the bees, the combs were removed and further incubated at 35°C for frequent sampling of the honey for analysis on chloramphenicol by LC-MS/MS.
The higher the concentration of chloramphenicol in the wax, the more residues were found in the honey. The highest concentration of chloramphenicol present in the honey was 0.3, 3.7 and 21.6 µg/kg, respectively. Taking into account the weight of the wax foundation and the honey stored into the comb, the transfer of chloramphenicol from beeswax to honey was 0.40, 0.36 and 0.21% (mean of all samplings), respectively. Hence the purchase and the use of contaminated wax foundations by the beekeeper can lead to concentrations above the MRPL of 0.3 µg/kg of chloramphenicol in honey (Commission Decision 2003/181/EC).

The specific chemical profile of Maltese propolis and its antimicrobial activity

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Seventeen Maltese propolis samples were studied by GC-MS after silylation. They demonstrated the typical Mediterranean chemical profile, rich in diterpene compounds: 32 individual diterpenes were identified, 22 of them were present in each specimen. The other abundant compound group was that of sugars and sugar derivatives. In some samples, however, another compound group was observed; the corresponding mass spectra were consistent with sesquiterpenyl esters of substituted benzoic acids. Two new propolis constituents of this group, daucane diterpene esters of hydroxybenzoic acids, were isolated. Their origin is suggested to be *Ferula communis*, as they are taxonomic markers for this species. All propolis samples were active against *Staphylococcus aureus* but only those with high concentration of terpenyl esters demonstrated antifungal activity against *Candida albicans.* The present results confirm that Mediterranean propolis is a valuable natural product with potential as an antimicrobial agent.

Bee products in human medicine - the need for standardization

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In recent years, medical problems such as pathogen resistance to antibiotics have caused a renewed interest in the medicinal use of the products of the bee hive, long used in folklore and traditional medicine, allowing them to take their place among the modern armoury of drugs. Whilst many scientific studies have shown efficacy and safety, many other claims that have been made lack scientific credibility. As with any drug of biological origin, a thorough understanding of the mode of action, and the standardisation of concentration of the active ingredients, together with standardisation of testing methods, are essential. The fact that reports of the efficacy of hive products are often anecdotal, and have hitherto been published in a wide array of journals, books and ephemeral literature, has arguably slowed the acceptance of the use of hive products in “conventional” medicine. The new journal the *Journal of ApiProduct and Apimedical Science* provides a single forum for peer-reviewed reports of research on the six main hive products of both *Apis* and non *Apis* bees: honey, pollen, propolis, wax, royal jelly, and bee venom. Authoritative review papers can also draw together the results of many studies, and provide a platform for the adoption of standardised techniques.

Characterization of a propolis extract from a French landscape mosaic marshlands/grove/forest

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It has been scientifically proven that propolis is a substance with a lot of biological activities. However,variability in its chemical composition, which is dependant of its geographical and botanical origins, is limiting its application for human and veterinary medicine. A solution could be to create a standardized propolis extract in order to ensure a chemical composition and consequently the eproducibility of its biological properties [Bankova, 2005]. With this end in view, a standardized mix of propolis was realized from the three principal
ecosystems constituting the mosaic landscape of the French region of Poitou-Charentes (Atlantic west coast).The polyphenolic composition of the extract has been determined qualitatively (by HPLC-MS) and quantitatively (by the Folin-Ciocalteu method and by Diode Array Detection). The first results revealed that the propolis extract presents an original and unique polyphenolic composition, with pinocembrin and galangin for main polyphenols. Furthermore, ten secondary polyphenols were highlighted and recent studies have shown particularly interesting biological activities for some of them. To conclude, for the first time, a standardized propolis extract from a mosaic landscape of the Atlantic west coast of France has been realized. A great part of the extract characterization has already been done, showing an original and unique composition, but now some experiments have to be made to complete it.

Theme III. BEE BIOLOGY and ECOLOGY:
Symposium 11. Nutrition and physiology in bees

Is there 'revers' food flow from brood cells to adults in honey bees?

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We investigated the likelihood of brood food consumption from larval cells by adult honey bees. Experiments were conducted using 4 three-frame observation hives. Every morning, 5 cells containing worker larvae at the age of 3-5 days were chosen and the brood food was stained with 2 µl indigo carmine solution (2.7%). Every bee inspecting such a stained brood cell longer than 3 seconds was carefully collected afterwards and its honey crop and midgut were examined for indigo carmine visually. In the evening, the combs containing the stained cells were removed and transferred to a host colony to prevent the study colonies from contamination with dye and to (conservatively) differentiate successful nursing and cannibalism. In total, 86 larvae were reared to the sealed stage by the host colony. Of 1367 adult bees inspecting these larvae, 3.6% (95% Confidence interval: 2.6-4.6%) showed staining with indigo carmine (7.1, 5.7,2.0 and 2.5% per colony, respectively), mostly in their honey crop. 98 larvae were cannibalized during the experiment or later in the host colony. Bees inspecting these cells during our experiment more likely (13.9%, 95% CI: 12.3-15.5%,n=1803) exhibited stainings in their honey crops (22.7, 14.1, 10.0, and 13.8% per colony, respectively). We randomly collected 652 control bees from the broodnest area of two colonies midway through the experiment as well as after the experiment and found only 1 bee stained with indigo carmine. Our experiment demonstrates a possible reverse food flow from intact larval cells to adult honey bees.

The impact of nutritional protein on the honey bee - a review

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Protein is a critical factor in the nutrition of larvae and imagines and of course for the whole superorganism, the colony.This is because malnutrition of larvae easily leads to cannibalism or low quality bees and malnutrition of nurses leads to weak larvae. In contrast to carbohydrates, the quality of proteinaceous food strongly differs depending on the season and the sources it comes from (kinds of pollen). Protein often is the limiting factor for the development of colonies.We review the reports about life expectancy of caged bees kept with different diets as well as impacts of various nutritional regimes on colonies and performance parameters as flight capability, life expectancy or broodcare behavior. During the last decades the necessity and possibility of artificial supplementation of proteins increased for various reasons. Artificial larval rearing and testing will become of greater importance. Therefore the requirements for artificial feeding of colonies, single bees and the demands of food to rear larvae *in vitro* are discussed.

Nutrigenomics in honey bees

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Malnutrition is a major factor affecting health, resistance to disease and survival in animals. In honey bees, pollen is the main source of dietary protein and contains essential amino acids for their physiological development while reducing their susceptibility to parasites and pathogens. However, the molecular mechanisms underlying the pollen nutrients impact on honey bee health and how it decreases the impact of parasites and pathogens remained to be determined. With the availability of the honey bee genome and high-throughput genomic tools, it is now possible to study the genome-wide influence. Therefore, the current challenge is to examine how nutrients in the pollen affect the expression of genes in the bee genome and identify the nutrient-influenced molecular pathways. For that purpose, we analyzed the influence of pollen nutrition at the molecular level in worker bees parasitized or not by the mites *Varroa destructor*, known for suppressing immunity and decreasing lifespan of bees. The effect of *Varroa* and pollen nutrition on bee transcriptomes will be presented and discussed at the meeting. Ultimately, nutrigenomics in bees promises to better understand how nutrition influences body homeostasis and may help reducing pathogen susceptibility.

Strategies of energetic and thermal optimization of foraging honeybees

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Foraging honeybees have to cope with an enormous heat loss because of their small body size, which calls for energetic optimization. The dependence of (flight) muscle function on thorax temperature, on the other hand, counteracts economic measures. We investigated the balancing of body temperature regulation (measured by thermography) with energetic investment (measured *via* CO_2 production, V_{CO2}) and solar heat gain in bees foraging sucrose from within a 12 ml flow-through respirometer chamber.
Bees gathering 0.5 M sucrose with unlimited flow in shade (<100 W/m²) regulated thorax surface temperature (T_{th}) at 40.5 °C at an ambient temperature (T_a) of 30 °C and at 37.2 °C at T_a = 20 °C. V_{CO2} amounted to 160 µl/min at T_a = 30 °C and to 175 µl/min T_a = 20 °C. Solar radiation (>500 W/m²) was invested to do both increase T_{th} and decrease V_{CO2}. However, this was done differently at different T_as. T_{th} increased by only 0.5 °C at T_a = 30 °C (to 41 °C) but by 3.3 °C at T_a = 20 °C (to 40.5 °C). This caused V_{CO2} to decrease to 110 µl/min at T_a = 30 °C and to increase to as high as 200 µl/min at T_a = 20 °C. With less profitable reward of 0.5 M sucrose at a limited flow of 0.9 ml/h, bees in shade decreased both T_{th} and V_{CO2} (to 37.5 °C and 36.7 °C, and to 70 µl/min and 135 µl/min, at T_a = 30 and 20 °C, respectively). Solar radiation increased T_{th} to 38.5 and 37.7 °C, and decreased V_{CO2} to 23 and 70 µl/min at these T_as.
Despite the differential regulatory strategies concerning T_{th} and V_{CO2} at different T_as and rewards, foraging in sunshine reduced energetic costs per stay at all T_as and reward rates (30-64%).
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Impact of transgenic proteins, Bt-pollen and colony affiliation on survival and longevity of honey bee workers

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The ecological and economic importance of pollination makes the honeybee a key test species in assessing possible adverse effects of genetically modified (GM) crops. Until now, there are no indications of adverse effects of GM crops on honeybees. Most of the published reports, however, are related to studies on first generation GM crops, expressing only a single insecticidal protein. Currently a new generation of multiple insect resistant crops challenges environmental risk assessment, because combinations of insecticidal proteins have to be tested yet. In this study, a total 1725 worker bees from six different colonies were exposed in oral toxicity tests to single or multiple Bt-proteins, to stacked Bt-pollen and to *Galanthus nivalis* (snow drop) agglutinin (GNA) at four different concentration levels to evaluate adverse effects of transgenic proteins on survival and longevity of honeybees. In the assays with purified transgenic protein feeding, dose response curves and LD 50 values were calculated. We discuss the results of our combined Bt-proteins or Bt-pollen on worker bee survival and longevity. In addition, for the first time our data clearly show the acute oral toxicity of GNA on adult honeybees. The estimated LD50 was in a similar range as observed in bumble bees and parasitoid wasps. We conclude that our results should be taken into account when commercialising transgenic GNA crops.

Does in vitro larval rearing conditions influence adults behavior?

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The utility of honeybee *Apis mellifera* is now established. Nevertheless hives health is damaged with pesticides usage. The influence of environment on the general functioning of a colony can be assessed through the behavioural comparison of different cohorts. The main objective of this study is to assess the effect of rearing conditions, in laboratory (*in vitro*) and in hive (*in vivo*), on flight
behaviour of adults. In this purpose 379 bees were tagged with individual chips stuck on thorax and a hive was equipped at its entry with a RFID (Radio Frequency identification Device) system. The bees were registered for 29 days. No significant difference between cohorts was observed for the age of the first flight, the last flight, and the number of lost bees per day. On the other hand all other tested variables like number and flight duration, the mean age of flying bees were significantly different between cohorts. The fact that some activity patterns were similar between cohorts, and the survey of *in vitro* bees during all the experiment time, make this results very promising for future research programs looking for studying influence of larval exposure to pesticides on adults behaviour.

Comparison of two methods to assess effects of insecticides on hypopharyngeal gland development of honey bee

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Hypopharyngeal glands (HPG) are the main organs responsible of royal jelly secretion. The size of the HPG is aged and food protein dependent, correlated to the amount of secretion, and the weight of the head. HPG development can be assessed with a microscope by measuring the acini diameter after dissection. This very useful method has some convenient: it requires dexterity to extract the gland, and the diameter of the acini is difficult to measure because of its pear shaped. In order to assess the HPG development, total protein of the gland can be measured with Bradford method, but it also requires extracting it from the head.The development of the HPG may be also affected by substances known for their insecticide effects like soybean tripsin inhibitor.The objective of this work is to compare two methods for assessing the effects of insecticides on HGP development. The first one consists in measuring the acini diameter, and the second one to measure the total protein of the head. The measurements are made on nurses intoxicated during 10 days with sublétal doses of dimethoate.

Theme II. DIVERSITY AND CONSERVATION:
Symposium 6. Drivers of bee
loss in Europe and impacts for society.

The effect of climatic variation on the mountain bumblebee fauna

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We surveyed the bumblebees fauna in a small area in Pyrenees-Orientales (SW. France) during 10 years. During this time, we observed very important variations of the species relative abundance. Some species that were once very abundant, as *Bombus lucorum* and *B. sylvarum*, could have been nearly absent of our samples other years. We also observed evident signs of local droughts and heatwaves. We hypothesised that the faunistic variations could have been related to these extreme climatic events. To test this hypothesis in first approximation, we established the correlations between the relative abundance of each species and several local climatic parameters. In the 13 bumblebee species tested, we observed that the relative abundance of 9 and the whole population density show a significant correlation with at least one climatic parameter. The cold and wet years have the highest diversity and population density, while the dry and hot years are correlated with the lowest density and diversity. Most species, as *B. lucorum* and *B. pyrenaeus*, seem particularly sensitive to drought while a few others, as *B. sylvarum* and *B. mesomelas*, seem to take advantage of these conditions.

Are droughts and heatwaves leading to local extinctions of bumblebees?

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It is now well known that a lot of bumblebees species are threatened in Europe and in N. America. Various hypotheses have been proposed to explain the regressions. Some of the hypothetical factors act at a continental level, as the general restructuration of the agriculture toward the use of synthetic nitrogen fertilisation, in place of leguminous. The landscape fragmentation is typically a local factor the spatial coalescence of which leads to large-scale effects. Since 2002, we observed a great number of situations where local droughts and heatwaves occurred in France, UK, Scandinavia, Turkey, leading to very strong local reductions of the bumblebees fauna. We observed so many local cases in 2007-2009 that we could hypothesise that a coalescence of these local effects could lead to a new general thread. Some species seem to be very sensitive to these risks: *B. lucorum* and *B. magnus* are noticeable cases.

Resource use of maize pollen by honey bees in differentially structured landscapes

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The expansion of maize cultivation in Europe raises the question of its role as protein source for honeybees. Here we analysed, maize pollen foraging distances as well as maize pollen intake of honeybee colonies in relation to surrounding maize field supply, using a replicated experimental approach on a landscape scale. We compared twelve complex agricultural landscapes with an increasing proportion of maize. A honeybee colony was placed in the centre of each landscape at the beginning of the maize pollen shedding period. Pollen traps were used to record the bee collected pollen spectrum. In addition, four rotating observation hives were used to record bee dances of maize pollen foragers in the different landscapes. A total of 622 bee dances were observed and decoded to assess foraging distances and locations.Overall mean foraging distance for maize pollen was significantly shorter as for non-maize pollen. There was no correlation between the proportion of maize fields in the surrounding landscapes and the proportion of maize pollen collected by the colonies. Overall mean maize pollen foraging was low, representing a minor proportion of the total pollen spectrum. Our data show that under the condition of a landscape with varying availability of alternative food resources the contribution of maize pollen to the protein diet of honeybees is of minor importance. Our results indicate that expanding maize cultivation may influence the pollen foraging ecology of honeybees.

Landscape complexity and flowering herbs enhance wild bee density and sweet-cherry yield

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Bee pollination enhances the production of many crops, and many farmers rely on pollination services provided by managed honey bees. However, honey-bee abundances are declining in many parts of the world and honey bees might not always provide the best pollination service possible. Wild bees could be an alternative, but little is known about their impact on yields and about what landscape-scale and local factors enhance their visitation rates in crops.We studied cherry pollination by wild bees and honey bees in eight commercial sweet-cherry orchards which were located in the centre of eight landscape circles with 1 km radius. Landscape circles differed in % semi-natural habitats. Although honey bees were twice as abundant as wild bees, cherry yield increased with tree visitation rates of wild bees, but not of honey bees. Wild bees were most abundant and yields were highest in landscapes with high % semi-natural habitats. In addition, yield was enhanced by high flower cover between the tree lines.Our results show that pollination services by wild bees in sweet cherry surpass pollination by honey bees. Farmers can easily attract wild bees into orchards by promoting flower resources between tree lines. However, farmers only can rely on wild bee pollination if semi-natural habitats are present in the surrounding landscape. We conclude that the conservation of semi-natural habitats, which provide nesting sites and additional food resources before and after cherry flowering, pays off, because it enhances gratis ecosystem services and consequently farmers` income.

Does bee decline matter? A framework for characterizing a pollinator's agricultural importance

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If it is true that bee decline is a matter of public priority, then it is important to be able to objectively characterize the extent to which agriculture depends on bees and other pollinators. The public conversation is frequently over-heated with hyperbole, and as an antidote I propose a conceptual model that provides a starting point for designing experiments on the importance of agricultural pollinators. The relevance of a candidate pollinator is contextual – in terms of (a) the reproductive responsiveness of a plant to the flower visitor and (b) the pollen vectoring competence of the visitor. These components, in turn, are each the product of at least two interacting dynamics, the plant's response to the flower visitor being the product of the plant's obligation to out-crossing and the morphological or temporal specializations of its flower which may invite or exclude pollinators, and the pollen vectoring competence of the flower visitor being the product of its potency to set fruit (% fruit set per single visit) and its availability (visits per minute). This framework accommodates related considerations such as the global increase in pollinator-dependent crops and the effects of parasites on bee foraging behavior.

***Apis florea* -the future dominant honey bee in Europe?**

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Apis florea is an open nesting dwarf honey bee native to Southern Asia. Three years ago the first *A.* florea nests were discovered in the Red Sea port cities of Aqaba, Jordan, and Eilat, Israel. *A. florea* is a very successful colonizer, even where conditions are less than favorable for this tropical bee, including sub tropical and semi-arid areas, and it has since established itself in the Aqaba/Eilat area. It swarms frequently and creates dense aggregations of colonies. Consequently, without deliberate intervention, its distribution is likely to spread from its current limited location to other areas in the Middle East, and along the Mediterranean coastline to Europe. Nectar and pollen resources are limited in many parts of the Middle East. The main risks of the invasion of *A. florea* are competition with *A. mellifera* and native bees for these pollen and nectar resources, and the potential for pathogen transfer. If we fail to eradicate the Aqaba/Eilat population soon, this invasion may become a major problem to Middle-Eastern and European beekeeping and native bee conservation. Our research goals are to: 1) develop a bio-economic model to assess the impact of this potential invasion to Israel's honey industry 2) devise specific traps/feeding stations for *A. florea* in order to monitor and eradicate its population by means of toxic baits

Bees in intensive cereal farming systems: landscape composition influences colony dynamics

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A five-year long study was recently initiated in western France to determine (i) how the composition of an intensive cereal openfield landscape influences honeybee colony development and performance and (ii)whether honeybees may be used as a model species to assess the management efficiency of natural habitat remnants. Fieldwork was undertaken in a 45000-ha long-term biological research facility where land use,farming practices and natural habitats are spatially referenced and documented in details over the past decade.Flower resources in this openfield landscape are mostly restricted to cultivated rapeseed and sunflower,available over short periods of time (in Mars-April and June-July, respectively). Therefore, we expect that the
seasonal food shortage spanning from early May to late June would exert strong constraints on colony dynamics and beekeeping activity, but that the presence of (semi-)natural habitat remnants in the proximity of apiaries may buffer those constraints by providing bees with alternative, steady-state, flower resources. Up to date, one hundred hives where assigned into 20 apiaries, randomly distributed over the study area, andmonitored biweekly using common population indicators: number of workers, brood area, hive weight and pollen quantity and species composition. Pollen analyses are carried out on pellets retained by pollen traps at hive entries. After two years of measurements, strong correlations could be established between some
indicators of population dynamics on one hand, and some major landscape characteristics on the other hand.The results will be presented and discussed in relation with a contemporary research program on sustainable agricultural systems.

Novel management to boost bee habitat quality in existing grass buffer strips

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Insect pollinators play a key role in crop and wildflower pollination but have suffered global declines, partly as a result of agricultural intensification and the associated habitat loss and fragmentation. Across Europe various agri-environmental schemes have been initiated with farmers offered financial incentives for sympathetic land management. In the UK a popular option is the establishment of sown grass buffer strips, with ~29,000 ha currently. However, typically they are floristically poor and as such offer limited biodiversity value. Introducing a sown wildflower component has the potential to dramatically increase the value of these buffer strips for many species, including bees. This study investigates management practices that aim to promote the establishment and persistence of wildflowers in existing buffer strips. The effectiveness of two methods was tested, individually and in combination, to increase the establishment of wildflowers for the benefit of bee species. The management practices were: (1) the application of a selective graminicide (fluazifop-P-butyl) which reduces the dominance of competitive grasses; (2) scarification to create germination niches into which wildflower seeds were sown. Responses of wildflowers, honeybees and bumblebees were monitored for two years after establishment. Preliminary results indicate that the combination of scarification, sowing and graminicide resulted in the greatest establishment and floral abundance of sown wildflowers, and the highest abundance of honeybees, and abundance and diversity of bumblebees. Further assessments in years three and four will determine if the sown wildflowers can persist and continue to provide habitat to support bee populations.

Theme III. BEE BIOLOGY and ECOLOGY:
Symposium 10. Mechanisms of Learning and Memory in Honey Bees

Mechanisms of reward and punishment learning in honey bees

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Biogenic amines are characterized in pathways evaluating reward and punishment, resulting in appropriate aversive or appetitive responses of vertebrates and invertebrates. Although antagonistic and complementary actions of these chemicals in regulating simple behaviors such as fight-or-flight response is known, there is a gap in our understanding of interaction of these biogenic amines in learning.
I report studies where we utilized the honey bee model and a newly developed spatial passive avoidance conditioning assay to probe potential integration of reward (octopamine: OA) and punishment (dopamine: DA) pathways in behavioral regulation. In this new protocol bees associated a spatial color cue with mild electric shock punishment. After a number of experiences with color and shock the bees no longer enter the compartment associated with punishment. Intrinsic aspects of passive avoidance conditioning are associated with natural behavior of bees such as punishment (lack of food, explosive pollination mechanisms, danger of predation, heat, etc.) and their association to floral traits or other spatial cues during foraging.
The results show that DA reduces the number of punishments received whereas OA increases the number of punishments received. These effects are dose-dependent and specific to the acquisition phase of training with no effect on short-term memory. The effects during acquisition are specific as shown experiments using the antagonists Pimozide and Mianserin for DA and OA receptors, respectively. This study demonstrates the integrative role of biogenic amines in regulation of complex behaviors in the honey bee as modeled in a novel non-appetitive passive avoidance learning assay.

Optic flow informs distance but not profitability for honey bees

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How do flying insects monitor foraging efficiency? Honey bees (*Apis mellifera*) use optic flow information as an odometer to estimate distance travelled, but here we tested whether optic flow informs estimation of foraging costs also. Bees were trained to feeders in flight tunnels such that bees experienced the greatest optic flow *en route* to the feeder closest to the hive. Analyses of dance communication showed that, as expected, bees indicated the close feeder as being further, but they also indicated this feeder as the more profitable, and preferentially visited this feeder when given a choice. We show that honey bee estimates of foraging cost are not reliant on optic flow information. Rather bees can assess distance and profitability independently and signal these aspects as separate elements of their dances. The optic flow signal is sensitive to the nature of the environment travelled by the bee, and is therefore not a good index of flight energetic costs, but it provides a good indication of distance travelled for purpose of navigation and communication, as long as the dancer and recruit travel similar routes. This study suggests an adaptive dual processing system in honey bees for communicating and navigating distance flown and for evaluating its energetic costs.

Some issues in the cognitive interpretation of invertebrate behavior

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Methodological and theoretical issues associated with cognitive interpretations of invertebrate behavior are discussed. Issues include the lack of behavioral taxonomies, inconsistencies in the definitions of behavioral phenomena, few examples of individual data, and lack of integration between the data gathered by experimental psychologists interested in invertebrate behavior and those in other fields.

Modification of olfactory learning and memory induced by siRNA targeting nicotinic acetylcholine subunits in the honeybee

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Acetylcholine is the major excitatory neurotransmitter in the central nervous system of insects and targets the numerous nicotinic acetylcholine receptors (nAChRs). The recent honeybee genome sequencing described 9 α and 2 β nicotinic subunits that can co-assemble following multiple combinations to form several nAChR subtypes.
The 11 subunits are expressed in the brain and two of them, α7 and α8 are found in structures involved in olfaction and memory processes. The role of these nicotinic subunits in olfactory learning and memory was studied using siRNA to induce partial silencing of their expression. Honeybees injected in the entire brain with α7 siRNA before multiple-trial olfactory learning presented poor performance during acquisition and memory tests compared to control animals. α7 siRNA injected before the retrieval tests had no effect on performance, excluding an involvement of α7 in retrieval processes. This result also indicated that olfactory perception was not depending on the presence of α7 subunit. α8 siRNA injected in the entire brain leaded to a retrieval impairment, an effect that can be reproduced by injection into the a lobes and not the calyces of mushroom bodies and that was not observed after injection into the antennal lobes.
As a conclusion, nAChRs containing α8 subunit must be involved in retrieval processes whereas nAChRs containing α7 subunit must be involved in acquisition processes. As each subunit deletion induced different effects on olfactory learning and memory, both subunits must be part of two nAChRs subtypes which would be not involved in the same cognitive processes.

Foraging prefence and flower handling in honey bee subspecies

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Foraging behavior of *Apis mellifera caucasica*, *A.m. carnica* and *A.m. syriaca* in Turkey was studied for intrinsic subspecies-based differences. Models of forager flower-color fidelity, risk sensitive behavior and maximizing net gain were tested. Foragers were presented artificial flower patches containing blue, white and yellow flowers. Some bees of each subspecies showed high fidelity to yellow flowers, while others favored blue and white flowers. The degree of fidelity, however, differed among subspecies and was dependent upon which color was favored. Bees of all subspecies demonstrated risk indifferent behavior regardless of whether they favored yellow flowers or blue and white flowers. Flower handling time differed among subspecies and increased with reward quantity, and when a reward was present. Flight time between consecutive flowers also differed among honey bee subspecies. Foragers of all subspecies had a higher net gain when visiting flowers with consistent rewards.

Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex

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Insects have contributed greatly to our understanding of associative learning because they allow learning protocols to be combined with experimental access to the nervous system. In the honeybee Apis mellifera, appetitive conditioning, in which bees learn to associate different sensory cues with a reward of sugar solution, has been the main tool for the study of learning and memory. Up to now, no study explored aversive learning in bees in such a way that simultaneous access to its neural bases is granted. Using odorants paired with electric shocks, we conditioned the sting extension reflex, which is exhibited by harnessed bees when subjected to a noxious stimulation. Bees learned to associate odorant presentations with an electric shock and extended afterwards their sting in response to the odorant previously punished. Absolute and differential conditioning procedures yielded significant acquisition performances, which were affected by variables such as interstimulus (ISI) and intertrial interval (ITI). Aversive learning leads to long-term memories that can be retrieved at 24h,48h and 72h after the last acquisition trial. The latter require translation and transcription as shown by pharmacological experiments. Since in our protocol bees extend the sting in response to the shock instead of showing an avoidance response, we asked whether bees trained in this way would actively avoid the punished odorant in an operant context. We show that after odor-shock conditioning, a bee placed in a Y-maze presenting the punished odorant significantly avoids that odorant, a fact that demonstrates the aversive nature of the associations learned.
In a further experiment, we trained bees with a double association, appetitive and aversive. Bees had to learn to extend the proboscis to one odorant paired with sugar solution and the sting to a different odorant paired with electric shock. Bees efficiently learned this double task, thus showing that they can master both appetitive and aversive associations simultaneously. While octopamine has been previously shown to substitute for appetitive reinforcement in bees, we demonstrated that blocking of the dopaminergic, but not octopaminergic, system suppresses aversive learning. Thus, mastering appetitive and aversive learning simultaneously is possible because the underlying reinforcement systems, the octopaminergic and the dopaminergic system, act as insulated modules preventing interferences between associations of different nature. These results show that aversive learning in honeybees can now be accessed both at the behavioral and neural levels, thus opening new research avenues for understanding basic mechanisms of associative learning and memory.

Operant conditioning protocols in honey bees

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Not surprisingly, as learning may be very useful during foraging, the ability to learn has been shown to be well developed in honey bees. Among learning mechanisms, conditioning plays a central role. Until now, the proboscis extension response protocol has been the center of most conditioning studies. Unfortunately, other learning mechanisms have received only little attention. We present a protocol to study operant conditioning in bees and propose several examples of application.

Olfactory learning in honey bees

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Learning and memory in honey bees has now been studied for over a century, beginning with the pioneering work of Karl von Frisch. Much of this work has centered on field studies of foraging behaviors related to identification and collection of nectar and pollen resources need for a colony's survival. It is now well established that honey bees from different paternal lineages within a colony show different genetic predispositions to learn. In recent decades honey bee learning ability has been very well studied in the laboratory using Proboscis Extension Response conditioning. This laboratory-based protocol has allowed many investigators to establish molecular and neural processes that underlie learning abilities in honey bees. Thus many pieces to the puzzle for a comprehensive understanding of learning are in place. In this talk I will argue that we now need ways to map knowledge for studies of learning in the laboratory to an understanding of how that learning ability applies under more natural conditions.

Theme V. OPEN SESSIONS:
Symposium 15A. Open Session

Preliminary results from a study on Balkan honey bees' genetic variability using isoenzymic approach

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The genetic variability of honey bee populations from eleven different regions of Bulgaria, Greece, Serbia and Montenegro has been studied using isoenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. All loci, were found to be polymorphic in most of the populations studied. Four alleles were detected at MHD-1 locus (MDH^{65}, MDH^{80}, MDH^{100} and MDH^{125}), three alleles at Me locus (ME^{90}, ME^{100} and ME^{106}), six alleles - at EST-3 locus (EST^{80}, EST^{88}, EST^{94}, EST^{100}, EST^{105} and EST^{118}), three alleles - at ALP locus (ALP^{80}, ALP^{90} and ALP^{100}), two alleles at PGM locus (PGM^{100} and PGM^{114}) and four alleles at HK locus (HK^{87}, HK^{100}, HK^{110} and HK^{120}). There was found, that ME^{100} allele was fixed in the Serbian populations and EST^{100} allele - in one of the Greek populations studied. The observed and expected heterozygosities (H_o and H_e) ranged from 0.161 to 0.272 and 0.222 to 0.306, respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, which was found to range between 0.001 (between one population from Serbia and one from Montenegro) and 0.101 (between one population from Serbia and one from Greece). The estimated mean F_{ST} value from allozyme data was 0.094.
Neighbor-Joining phylogenetic tree and UPGMA dendrogram were obtained by genetic distance matrix methods; populations studied are grouped in two clades. The populations from Bulgaria and Greece were clustered in the first clade and these from Serbia and Montenegro – in the second one. The research is in progress including other Balkan countries.

Breeding and conservation projects in Italy: economy and science co-operate to save biodiversity

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The Italian bee research unit CRA-API is responsible for coordination of breeding activities inside the National queen breeders registry, which was set up by Ministerial Decree in 1997. The aim of the registry is to protect and improve the native Italian races *Apis mellifera ligustica* and *A. m. siciliana* (the latter badly endangered of extinction by hybridization with non-autochtonous bees). The research unit organizes performance testing, anonymous distribution of queens, courses for testers, data collection, biometric and genetic analysis. The traits currently screened in routine performance testing are honey production, docility, swarming tendency and, to a lesser extent hygienic behaviour. Breeding values are calculated according to the modified BLUP method by the Hohen Neuendorf Bee Institute (Germany). Results from 7 years of application of this method show a general increase in breeding values for most of the considered traits.Furthermore, besides ongoing efforts for *A. m. siciliana* conservation in Sicily, in the wake of the recent interest by Slow Food international foundation for Biodiversity that provides new incentive to local beekeepers for using the autochtonous bee, a new reintroduction project is underway. Once the reintroduced population is established routine testing will be performed as for *A. m. ligustica* and the collected data will be used for implementation of the BLUP method in a specific database for *A. m. siciliana*.

Analysis of the relation between pathogens and the loss of bee colonies (results 2008-2009)

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In Poland, the problem of massive losses of bee colonies occurred in the autumn of 2007. From autumn 2007 to spring 2008 Polish apiaries lost on average about 30% of their colonies, while in the period between autumn 2008 and spring 2009, the average national loss of bee colonies was lower and did not exceed 10%. However, in both years, in many apiaries losses were much higher than the national average.In 2008 and 2009, samples from 220 apiaries with the loss of colonies at the level of between 30% and100% were taken for laboratory tests. The size of the losses were calculated for each apiary individually, based on the number of wintered colonies in relation to the number of colonies prepared to winter in the autumn. Bee samples were collected from several, randomly selected dead colonies (on average from 7) and individually examined for the presence of *V.destructor*, *Nosema* spp. and viruses: ABPV, CBPV, IAPV, DWV. In order to determine the pathogens' impact on the extent of bee colonies losses in different apiaries and the relationship between individual pathogens, statistical analysis of test results for 1000 colonies was performed (142 apiaries, in each at least 5 colonies were examined).
Linear regression analysis was carried out in order to investigate the following:
- the impact of the number of pathogens found in the surveyed bee colonies on the size of losses that occurred in each apiary
- the relationship between the level of *V.destructor* infestation and the magnitude of losses- the relationship between the level of *Nosema* spp. infestation and the magnitude of losses
- the relationship between the *V. destructor* and *Nosema* spp.
- the relationship between the level of *V.destructor* infestation and the presence of viruses
- the relationship between the level of *Nosema* spp. infestation and the presence of viruses

Territorial biodiversity and consequences on physico-chemical characteristics of collected pollen in bee colonies

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Pollen resources can become a constraint for honeybee in a cereal farming agrosystem and can impact the health of the bee colony. Pollen is an expensive product for beekeepers and there is no artificial food available today able to lead to a full development and protection of the insects. It appears useful to bring knowledge in bee ecology about cropping system capacity to provide food in quantity and quality throughout the year.
This survey was devoted to deliver information on (i) the flower range exploited in an agrarian environment in western France, (ii) the physico-chemical composition of the pollen supplied, and (iii) the territorial biodiversity foraged in different periods. The diversity of the collected pollen was studied by palynological analysis, whereas a land use monitoring was recorded. We carried on a complete nutritional component survey of these multifloral samples throughout a year about proteins, lipids, and glucids.A strong decrease in the pollen supply in the food shortage period in late spring, and a large amount of crop pollen in summer is observed. The succeeding contribution of different floral groups such as crop flowers, weeds and woods, clearly shows the influence of agricultural practices as a major food supply factor for pollinators.

Longitudinal comparative study of colonies headed by US and Australian queens in US East coast operations

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Packages of Australian bees were established on various comb types in February of 2007. Some of these packages were installed with the queen that accompanied the Australian bees (n=34), while others had their queen replaced (n= 37) with queens produced in Hawaii. Overall, only 26.8% of colonies survived over the entire 9 months of this study. While, there was no difference in the number of colonies surviving in the group managed for pollination as compared to those managed for honey production (χ^2=0.54, P=0.462), fewer AUS colonies survived (14.7%) as compared to US colonies (40.5%; χ^2=5.84, P=0.015). Incidence of disease, particularly the incidence of chalkbood, differed between the two groups. Survival rates of colonies established on various comb types also differed. Potential explanations for these differences will be discussed.

Origin of queens as a cause of colony losses

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Colony losses in Croatia are significant and ranged from 10 to 30% per apiary. Some beekeepers report losses up to 90%. Through the survey at 1591 apiary attention was given to the reason of loss. By the opinion of the beekeepers, the most common cause of loss was varroa (47%) followed by problems with queens (22%), nosemosis (15%), lack of food (15%) and other reasons (1%). In our research, attention was given to the source of queen and the incidence of loses. Queen breeding in Croatia has tradition and significant part of beekeepers introduces queens from known origin. However, part of beekeepers still relays on natural queen replacement through swarming or supersedure. From the total, colony losses due to queen problem were reported by 36,38% beekeepers in 2008 and by 27,49% beekeepers in 2009. The losses are indicated through the year. Losses of colonies are lower at the apiaries with regularly replaced queens.

Nectar flow of some sunflower hybrids

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Nectar production and sugar concentration of 17 selected sunflower hybrids used in Slovakia was evaluated in this study. The influence of the main climatic factors on the nectar production was studied as well. The experiment was carried out at two different sites in Slovakia with different climatic and soil conditions, in the Liptovsky Hradok and Nitra.
All hybrids of sunflowers studied produced nectar, although in relatively small amounts from 0,005 mg to 0,045 mg with sugar content variability from 30 to 60%. Average nectar production at the Nitra locality was 0,028 mg, at the Liptovsky Hradok locality 0,0137 mg.

What is the relation between Israeli Acute Paralysis Virus and Colony Collapse Disorder? Utility of IAPV detection

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The worlwide population of *Apis mellifera* has decreased during the last decade. This late phenomena has been named as Colony Colapse Disorder (CCD) and it is suspected to be a multifactorial problem mainly caused by monoculture fields, pesticides and a wide variety of pathogens. In 2007, Israeli acute paralysis virus (IAPV) was described as indicator of CCD as it's presence in the analyzed CCD colonies was always confirmed (Cox-Foster et al., 2007).
Having this into account, the aim of this work was to analyze Spanish samples and evaluate the presence of IAPV and its relation with the occurrence of CCD. Once detected, a phylogenetic study was developed to determine the differences between the sequences of the positive samples and to understand the origin of the virus. In Spain only one colony from 484 was positive when analyzed by real time RT-PCR (Kukielka and Sánchez-Vizcaíno, 2010). Therefore, new samples were analyzed in order to look for more positive samples. As results, one sample coming from an asyntomatic colony was identified as positive. Once sequenced, the phylogenetic analysis showed differences with the isolate already described. This results show that IAPV is not related with the CCD occuring in Spain and there might be trade relationships with other countries that have IAPV. This conclusions could be really useful when establishing epidemiological surveillance programs in Spain.

A new method of assessing pesticides and repellents in honey bees

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The purpose of this talk is to help bridge the gap between studies of avoidance learning conducted within the frame work of traditional experimental psychology and studies of repellency conducted in the areas of biology and entomology. The argument is advanced that the psychology of learning has much to offer biologists and entomologists in the study of repellents. Links between signaled avoidance learning and repellents are described. New strategies to test putative repellents based on traditional avoidance and conditioned suppression paradigms are illustrated using Deet, citronella, and butyric acid.

Imidacloprid enhances outbreak of nosemosis in honey bees

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Imidacloprid is neonicotinoid used for dress seeding to protect plants against insects. We investigated the influence of sublethal doses of imidacloprid on outbreak of digestive disease of honey bees caused by *Nosema* sp. Four days old bees were caged and treated in two successive days by feeding honey solution with a) *Nosema* spores and imidacloprid (2ng/bee) b) *Nosema* spores and c) imidacloprid (2ng/bee) only. As a control served honey solution with DMSO (solvent for imidacloprid) and pure honey solution. Workers were either returned to the small colonies free of *Nosema* sp. in the experiment 1 or left in the cages and fed by inverted sugar solution in the experiment 2. Bees were examined for the presence of *Nosema* spores in the mid-guts after 11 days. Results indicated that bees treated with *Nosema* sp. and imidacloprid are infected more frequently compared to bees treated with infective *Nosema* solution only. Bees that received *Nosema* spores and imidacloprid were also first to disappear from the colony. This study showed combined negative effects of imidacloprid and *Nosema* sp. on honey bees and suggests that the sublethal doses of neonicotinoids could enhance nosemosis.

Do varroa mites and pesticides synergize to affect bee larvae?

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Two sets of honey bee (*Apis mellifera*) larvae reared in an incubator were fed a diet for two days *ad libitum* containing either 0.8 ppm chlorpyrifos, 200 ppm imidacloprid, 200 ppm amitraz, 100 ppm fluvalinate, 50 ppm coumaphos, 200 ppm mycobutanil, 200 ppm chlorothalonil, 200 ppm glyphosate, 200 ppm simazine, or diet alone. Pre-pupae resulting from the second set then were exposed to two varroa (*Varroa destructor*) mites for two days. All prepupae were screened for the expression of 46 candidate genes implicated in cellular and immune response, development, and presence of pathogen genes, along with two controls for transcript abundance (encoding actin and RPS5) using real-time PCR reactions. Both pesticide exposure and/or varroa mite parasitism on honey bee larvae led to changes in gene expression, specific for genes that were upregulated, down regulated or neutral. Transcripts levels for PGRPSC 4300, a pathogen recognition gene, increased in larvae exposed to varroa mites ($P<0.001$) and were not changed across pesticide treated larvae. Similarly, DWV transcripts increased substantially with exposure to varroa mites ($P<0.001$), but not with exposure to pesticides. Transcripts levels for PPOact, an immune end product, differed across pesticide treated larvae ($P<0.001$). Significantly higher expression of RNA for this gene was found in imidacloprid, amitraz, mycobutanil, and chlorothalonil treated larvae. Transcript levels for the storage protein AmHex70 decreased significantly after pesticide or varroa mite exposure ($P<0.01$). Down regulation effects also were seen for the gene encoding a cytochrome p450 member (CYP4g11), where the effect of mites was significant ($P<0.01$) in comparison to non infested larvae. The effect of chlorothalonil and varroa mites also was significant in downregulation of transcripts for this CYP. There were significant interactive effects between varroa exposure and pesticide exposure for numerous pesticides and variable genes, indicating a synergism between che mical insults and mite impacts.

Neonicotinoid toxicity towards bees

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The neonicotinoids, a class of insecticides of relatively recent concept, have been used since the '80s for the interesting effects that they could have being systemic and long lasting. However these active ingredients manifested also a high toxicity to pollinating insects, particularly against the honey bee, causing numerous secondary effects, often not easily identifiable, such as behavioural disturbances, impaired orientation, impairment of social activities, etc... In recent years there have been alarming phenomena of bee mortality, due, in some cases, to the use of neonicotinoids as tanning agents and as pesticides.
It was therefore considered appropriate to test in the laboratory the toxicity of Imidacloprid, Clothianidin and Thiametoxam on different strains of *Apis mellifera* to verify a greater or lesser sensitivity to the three active ingredients.
To this end, using the method developed at the DI.VA.P.R.A., commercial products were used. They were tested initially at the concentrations recommended for field treatments. Where observed mortality of the treated bees was higher than that found for the untreated control, decreasing concentrations were tested down to concentrations which had no longer significant differences compared to the controls. Observations and films were made during the experiment.
The bees that died during the experiment were collected and analyzed to detect the amount of active ingredient detectable; such amounts resulted much lower that administered quantities.

Type I and type II pyrethroids modify honeybee antennal neurons function

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Social structure in honeybees' colony relies on inter-individual chemical communication. Chemical communication is mediated by odors and pheromones detected by olfactory receptor neurons (ORN) localized in antennae. The colony collapse disorder has been described in many countries around the world and one of its main symptoms is the desertion of the hive by adult worker bees, leaving the queen with a small group of young bees. One hypothesis to explain this phenomenon would be the disruption of chemical communication by insecticides to which honeybees are exposed during foraging activity for instance. Recent studies have shown that several insecticides can also be found inside the hive. We used electrophysiology to study the mechanism of action of several pyrethroids insecticides, including permethrin, tetramethrin (type I) and deltamethrin (type II), on isolated ORNs. We focused here on the voltage-gated sodium channels embedded in ORNs plasma membrane and which underlie the normal electrical activity of neurons. Our results show that pyrethroids induce a tail current upon repolarization by slowing down the sodium channel closing. On average, 10 µM permethrin or tetramethrin modify 20 ± 4 and 22 ± 7 % sodium channels respectively. Dose-response curves allowed an estimation of an EC_{50} at 3.5µM for permethrin. The pyrethroid-induced tail current could be responsible for abnormal information processing in antennal olfactory receptor neurons and would lead to defective detection of odors and pheromones if *in vivo* reach sufficient concentrations.

Assessment and management of pesticide risks to bees- an ongoing scientific and legal challenge

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Risks to bees are the earliest recognized environmentally damaging side effects of pesticides. Over the years effective scientific approaches to predict the side effects of pesticides to honey bees have been developed. These assessments are now legally required in Europe and elsewhere. For the legal authorisation of their pesticides, industry should always provide ample evidence about the side effects on bees along the lines of scientific study legally prescribed.
Nevertheless, science, legislation, pesticides and their uses are developing continuously. The ICPBR Bee Protection Group – who developed the scientific structure of the current risk assessment and management in Europe – brings together all experts in Europe to keep up with these developments and to jointly advise about new solutions. The paper further elaborates on the scientific principles and current developments of the ICPBR assessment and management of pesticide hazards to bees.

Will it be possible use of *Apis mellifera* as a tool of certification for the evaluation of the environmental and agro-alimentary bio-security?

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Apis mellifera is an excellent environmental bioindicador of pesticides and other pollutants in according to scientific reporters. In fact tests of toxicity on bees to be able to classify and commercialize pesticides. The aim has been to study the possible use of *Apis mellifera* as a method of evaluation of the environmental and food-processing biosecurity in fruit and vegetable fields. Methodology has three phases. Firstly, two stations of monitoring (with two colonies of *Apis mellifera* each) were located in plums, peaches and tomatoes fields from a cooperative with integrated production in the province of Badajoz (Spain) and controlled according to a protocol previously stablished (a). In the second phase, the dead bees were gathered every 7 days during 5 months and those that overcame the threshold of mortality surrendered to chemical (b) and palinologic (c) analysis. Finally, results gave place to the elaboration of a biosecurity map by means of Geographical Information Systems, indicating type and origin of pollution. 40% of the samples overcame the threshold of mortality (250 bees died by station and week). In 100% of the above mentioned samples it was demonstrated the presence of different group of pesticides, such as Neonicotinoids (thiamethoxam, clotianidin and imidacloprid) and Organophosphates (fenitrotion, fosmet and clospirifos etile). . In conclusions, bees biomonitoring can be considered an useful and very effective method of evaluation and self-control to be used by farmers and a tool of certification for the future. This way, farmers might be allied and not enemies of bee-keepers.

A Methodology To Evaluate The Effects Of Pesticides To Honeybees: Recording Individual Behaviour Using Microchips

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Losses of foraging bees are sometimes attributed to alteration of the flight pattern between a meliferous plant treated with an insecticide and the hive. A limited number of studies have investigated the impact of pesticides on homing flight. This fact results from the difficulty of measuring the time between the food source and the hive. Monitoring the flights of the foraging bees necessitates their individual identification. Most of the monitoring techniques are limited by the number of bees monitored simultaneously and the time span during which observations can be made. However, techniques of automatic tracking and identification of individuals have the potential to revolutionize the ecotoxicological approach of behaviour. Radio Frequency Identification (RFID) seems to offer the most advantages (unlimited number of codes, large number of simultaneous recording, quick reading and through materials such as wood). We have developed a method under tunnel to study the effects of insecticides on the life-history traits of foragers. Here, we present the results obtained with fipronil. The strengths and weaknesses of our approach are discussed.

Theme III. BEE BIOLOGY and ECOLOGY:
Symposium 8. Bee genome and genomics

Insights into the molecular evolution of sex determining genes in corbiculate bees

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One important question in genome evolution is how the origin of a new gene function influences the evolution of other genes in the genome. An interesting system to study this are duplicated genes, that evolved under the model of neofunctionalization in which one copy gains a function not present in the progenitor gene and the other copy retains its function. The sex determination system of the honey bee *Apis mellifera* provide an interesting case to study this general question and to decipher the evolutionary forces that have been acted in the past. The initial signal of the sex regulatory pathway in honey bees, the *complementary sex determiner* (*csd*) gene, arose by a recent duplication from the ancestrally conserved progenitor gene *feminizer* in the honey bee lineage. Both genes are involved regulating the sex determination process but evolved under contrasting selection regimes. After the duplication, *csd* underwent strong positive selection and high numbers of different alleles are maintained over extended period of time by balancing selection. The analysis of synonymous and nonsynonymous changes over evolutionary time windows between *fem* of six bee species show that after the rise of *csd* in honey bees *fem* underwent stronger selective constraints as it does in species not possessing *csd*. The results suggest that interference of *fem* with *csd* during the rise of the *csd* allelic mechanism has substantially shaped *fem* nucleotide sequence evolution. These findings have more general implications for our understanding of the evolution of the genome´s gene repertoire

Evidence for a genetic basis of shift work in honey bee pollen foragers (*Apis mellifera* L.)

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Division of labour is a fundamental property of insect societies as well as human societies. The specialisation of different individuals in different tasks increases the overall work performance and efficiency and is the very foundation of the evolutionary success of social insects and human societies. In human societies an advanced form of division of labour, especially since the industrialisation, is shift work, where individuals perform the same task but in subsequent cohorts in time. Such shift work has not been documented in colonies of social insects so far. Here we used foragers of two honeybee (*Apis mellifera*) colonies and microsatellite genotyping to test whether a time preference exits in foraging honeybees. We found that the patriline identity of the foragers significantly influences the threshold of individual workers to forage either in the morning or evening. Thus individual foragers differ in their preference for the “early” or “late” shift, resulting in shift work like division of labour in the colony.

Evolution of antimicrobial peptides in bumble bees

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Coevolutionary arms races between hosts and parasites (e.g. nematodes, protozoa, microsporidia, bacteria and viruses) seem to be one of the driving forces having a significant impact on components of the genome. Selection on insect immune system genes might act on genetic variation to escape pathogen attack. Selection might act on genes related to the innate immune system, as could be shown for *Drosophila.* However, high pathogen loads in social insects may drive the rapid evolution of immune related genes (e.g. antimicrobial peptides (AMP), immune pathway genes) compared to non-immune related genes. Comparative studies of amino acid composition of immune genes between species of the same genus may indicate the type of selection (measured as the ratio of synonymous vs. non-synonymous substitutions) acting on those genes. To study the selective pressure acting on the immune system we sequenced three AMP (abaecin, defensin and hymenoptaecin) genes of eight different bumble bee species (N=24). Variable amino acids are not randomly distributed along the peptide, as variable sites are limited due to peptide domains or conformational constraints. Highest variability is found for defensin and abaecin with 19/87 and 7/40 amino acid changes, respectively. Hymenoptaecin showed less variability with 10/91 changes in amino acid composition. In all cases within-species diversity is low compared to between-species diversity.

QTL - Mapping of larval Varroa resistance in honeybee drones (*Apis mellifera*)

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The ectoparasitic mite *Varroa destructor* is a major threat for beekeeping world wide. In order to avoid chemical treatment several selection experiments have been carried out in the past resulting in *Varroa* tolerant stock of mostly unknown resistance mechanisms. We conducted a QTL-mapping on drone offspring of hybrid sister queens resulting from a natural cross between queens from the *Varroa* tolerant Gotland population (Fries *et al.*, 2006) and presumably susceptible drones from an unselected German population. We focused on individual larval resistance in drone pupae by suppression of mite reproduction, which has been shown to be rather a trait of the host than of the mite, since colony resistance traits are very complex and difficult to study on the molecular level. A major advantage of using haploid drones rather than diploid workers in a mapping approach is the simple genetic background in the mapping population with only maternal segregating alleles. Because interactions between homologous alleles are impossible in haploids, alleleic impact on the phenotype is expected to be much more explicit. Covering the genome with over 2000 microsatellite markers and two mapping populations with over 300 drones, we identified three candidate regions with potential major QTL for larval *Varroa* resistance. The identification of resistance alleles will allow for swift selection of target resistance alleles in future breeding programmes for *Varroa* resistance in the honeybee.

Honey bee thermal/chemical sensor, AmHsTRPA, reveals neofunctionalization and loss of TRP channel genes

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Insects are relatively small heterothermic animals, and thus they are highly susceptible to changes in ambient temperature. However, a group of honey bees (*Apis mellifera*) is able to maintain the brood nest temperature between 32-36°C either by cooling or by heating the nest. Nevertheless, how honey bees sense the ambient temperature is not known. We identified AmHsTRPA, a honey bee Hymenoptera-specific TRPA (HsTRPA), channel which is activated by heat with an apparent threshold temperature of 34°C and insect repellents such as camphor *in vitro*. *AmHsTRPA* is expressed in the antennal flagellum, and ablation of the antennal flagella and injection of AmHsTRPA inhibitors impair warmth avoidance of honey bees. Gustatory responses of honey bees to sucrose are suppressed by noxious heat and insect repellents, but relieved in the presence of AmHsTRPA inhibitors. These results suggest that AmHsTRPA may function as a thermal/chemical sensor *in vivo*. As previously shown, Hymenoptera has lost the ancient chemical sensor, TRPA1; however, AmHsTRPA is able to complement the function of *Drosophila melanogaster* TRPA1. These results demonstrate that HsTRPA originally arisen by the duplication of *Water witch* has acquired the thermal and chemical responsive properties, and this has resulted in the loss of ancient TRPA1. Thus, this is an example of neofuntionalization of duplicated ion channel gene followed by the loss of the functionally equivalent ancient gene.

Brood-dependent plasticity in behavioral and molecular circadian rhythms in bees

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The social environment influences the circadian clock of diverse animals, but little is known on the functional significance, the specifics of the social signals, and the dynamics of socially-mediated changes in the clock. Worker bees and ants naturally switch between activity with and without circadian rhythms along with their role in the division of labour that organizes their societies. Foragers have strong behavioral and molecular circadian rhythms, whereas "nurse" bees typically care for the brood around the clock with no circadian rhythms in behavior or clock gene expression. We found in honey bees that nurse-age workers that were restricted to a broodless comb inside or outside the hive showed robust behavioral and molecular circadian rhythms. By contrast, young nurses tended brood with no circadian rhythms in behavior or clock gene expression, even under light-dark illumination regime or when placed with brood in a small cage outside the hive. This behavior is context-dependent because nurses showed circadian rhythms in locomotor activity shortly after removal from the hive, and in clock gene expression after ~16 hrs. These findings suggest that direct interaction with the brood modulate the circadian system of worker honey bees. The dynamics of rhythm development support a model positing that at least some pacemakers continue to oscillate and be entrained by the environment in nurses that are active around the clock. These cells set the phase to the clock network when the nurse is removed from the hive. Because it has been suggested that sibling brood care evolved from maternal behaviour, we tested whether bumble bee (*Bombus terrestris*) queens that care alone for their first batches of offspring, are also capable of around-the-clock activity. We found that gynes typically emerged with no circadian rhythms, but later in life showed robust rhythms. Mating and diapause did not affect the expression of circadian rhythms, but colony-founding queens with brood showed no, or only attenuated circadian rhythms. By contrast, queens for which we removed the brood or that lost it for other reasons, switched to activity with strong circadian rhythms. This remarkable plasticity in queens is consistent with the hypothesis that task related plasticity in the circadian system of workers evolved from maternity-related plasticity in the circadian clock.

Genotype- environment interactions in *Apis mellifera ligustica*

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Genotype–environment interactions (GxE) are the effect on the phenotype of interactions between genes and the environments in which a genotype is placed. GxE are common in many traits and are well known by animal and plant breeders.
Recent studies of GxE in honey bees have focused on the individual level while there are few studies at the colony level. Although population genetic studies have shown that *A. m. ligustica* in Italy resembles one large population, probably as a result of large scale commercial queen trade, reports from beekeepers and professional breeders pointed to the existence of different reactions of the same genotypes in different Italian environments. Thus, the aim of this study was to assess whether, in a country with many different environments but with large scale commercial breeding and a long tradition of selection towards general productive superiority, there are still locally adapted honey bee populations. To verify the existence and magnitude of GxE interaction in honey production and spring development of Italian honey bees, three *A. m. ligustica* subpopulations were compared in three habitats differing in flora and climate.
The results from a total of 165 colonies showed significant interactions for both considered traits. Interestingly, for two of the considered origins, it was clear that colonies produced most when kept in their region. This study will provide useful information for national coordinated breeding programs, in terms of choice of testing stations but also for regional agricultural development policies.

Carniolan honeybee (*Apis mellfera carnica*) conservation in local geographic area

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Breeding and selection of indigenous *Apis mellifera carnica*, is supported by the EU project and Slovenian Ministry of Agriculture Forestry and Food in order to support local bee breeding activities. Beekeepers in Bela Krajina have decided to preserve their local population of Carniolan bees. In 2008 they started to organize and conducted conservation measures, by involving hobby and commercial beekeepers to participate in the project. Their aim supported by the local consent is to explore local honeybee colonies for selection, to establish secure mating area and to organize controlled mating station. Honeybee colonies from participating apiaries in the project, were sampled and analysed with 24 DNA microsatellite loci, further wing venation, behaviour characteristics and bee coloration were evaluated. The results of characteristics were analysed in order to select queen mother colonies and drone colonies exposed to the mating station. Colonies from 22 apiaries showed potential morphological and ethological qualities and minimal hybridisation were considered for further breeding. Selection and rearing quality queens in accordance with the project is conducted to replace dead colonies from different reasons and to repopulate introgressed colonies.

THURSDAY 09:20 – 12:20 / HALL C
Theme V. OPEN SESSIONS: Symposium 14. BeeDoc presentation

Bees in Europe and the Decline of Honeybee Colonies (BEEDOC)

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The BEE DOC comprises a network of eleven partners from honeybee pathology, chemistry, genetics and apicultural extension aiming to improve colony health of honeybees. The BEE DOC will empirically and experimentally fill knowledge gaps in honeybee pests and diseases, including the 'colony collapse disorder' and quantify the impact of interactions between parasites, pathogens and pesticides on honeybee mortality. Specifically BEE DOC will show for two model parasites (*Nosema* and *Varroa* mites), three model viruses (Deformed Wing Virus, Black Queen Cell Virus, Israel Acute Paralysis Virus) and two model pesticides (thiacloprid, τ-fluvalinate) how interactions affect individual bees and colonies in different European areas. The BEE DOC will use transcriptome analyses to explore host-pathogen-pesticide interactions and identify novel genes for disease resistance. The BEE DOC will specifically address sublethal and chronic exposure to pesticides and screen how apicultural practices affect colony health. The BEE DOC will develop novel diagnostic screening methods and develop sustainable concepts for disease prevention using novel treatments and selection tools for resistant stock. The BEE DOC will be linked to various national and international ongoing European, North- and South-American colony health monitoring and research programs, which will not only ensure pan-European but also global visibility and the transfer of results to apicultural practice in the world community of beekeepers.

Report of the activities of the BEEDOC 'Diagnostics department

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The author will report on the activities of the BEEDOC ‘Diagnostics department’. The BEE DOC will develop three levels of diagnostic tools. A ‘research grade’ tool will be an extension to the quantitative-PCR array BEE PATH developed by Dr. Jay Evans (Beltsville, USA) [Journal of Invertebrate Pathology 2007, 93:135-139], which uses 48 honeybee and pathogen genes in parallel. We will extended this array, by completing the list of pathogens and including novel genes provided by WP3 and WP4 and will rely on a colorimetric microarray technology. An ‘extension grade’ diagnostic tool will be based on simple PCR based protocols. For example Multiplex Ligation-dependent Probe Amplification (MLPA) is widely used to screen for various genetic applications. Finally we will develop a ‘field grade’ diagnostic tool for viral infections to be used by the beekeeper. These so-called Lateral Flow Device will be based on qualitative immuno-chromatography.

Bee Doc: novel treatments for the control of honey bee diseases

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The BEE DOC project aims to develop novel treatments for treating honeybee diseases by using therapies designed by evolution and the honeybee themselves. I will overview the range of such novel treatments, then I will focus on three themes that BEE DOC will develop. 1. Lactic acid bacteria (LAB) from the genus *Lactobacillus* (and *Bifidobacterium*), that are known as favourable bacterial species, commonly found in healthy individuals and commercially important through their use in probiotics (live micro-organisms, which confer health benefits on the host). The honey stomach of the honeybee has a unique probiotic gut flora with *Lactobacillus* and *Bifidobacterium* bacteria that have evolved in mutual dependence on one another. LAB exist in a nutrient rich niche whereas honey bees are protected by the LAB from harmful micro-organisms, including AFB. LAB produce such antibacterial compounds as organic acids, hydrogen peroxide, diacetyl, benzoate, and bacteriocins, all of which are beneficial for humans and animals and presumably for honeybees as well. 2. Compounds from propolis collected by bees, tested for their impact on disease control and prevention. Honeybees are not unique among social insects in defending their colony from microorganisms with plant secondary compounds, but their use of propolis is particularly well developed. 3. Honeybee-produced peptides that have antibiotic properties. These will be produced within BEE DOC by recombinant technology and applied to the colony for disease treatment. Finally, BEE DOC will develop a web-based interactive treatment tool to aid beekeepers in deciding when and how to treat honeybee diseases.

Bee Doc Prevention Department

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To prevent honey bee colony losses, background information on pathogen impact and interaction effects between pathogens and pathogens and the environment is needed. We have studied interactions between the *Nosema* parasites (*Nosema apis* and *Nosema ceranae*) and found no clear individual level competitive advantage between the two parasites. The dominance of *N. ceranae* in parts of the world remains an enigma. Ongoing surveys of *Nosema* prevalence suggest regional differences. Work on *Varroa* mite tolerance characteristics in colonies surviving mite infestations suggest that suppressed mite reproduction is important for mite tolerance. Bee Doc also studies the effects of probiotic bacteria as natural defence systems in honey bees. Indications are that the composition of the gut microbiota is of great importance for honey bee health.

The structure and function of antimicrobial peptides in defence of honeybee against microbial pathogens

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Antimicrobial peptides (AMPs) are essential components of host defences against infectious microorganisms. In animals they have been implicated in three alternative defensive systems: one is defined by the immediate upregulation of genes encoding AMPs, another is characterized by the inducible systemic release of AMPs from cellular reservoirs and the third

alternative is the systemic constitutive production of AMPs. As example of the third class of AMPs are royal jelly (RJ) peptides royalyzin - bee defensin and apisimin. Insect defensins share a high molecular identity and form a structure comprising a a-helix and two b̃strands, stabilized by three disulfide bridges. We have described an antifungal activity of honeybee royalisin and its antibacterial activity against the honeybee pathogen *P. larvae*, the cause of serious disease of honeybee larvae, American foulbrood (Bíliková et al. *Apidologie, 32*, 275-28, 2001). AMPs possess direct bacterial killing properties, partly by disintegrating bacterial membranes, and some also inhibit functions of intracellular biopolymers. The native AMPs upregulate the host defense, as chemoattractants or having various additional effects, like RJ peptide apisimin have both antifungal activity and stimulatory effect on production of cytokine TNF-a (Šimúth et al. *J. Agric. Food Chem*., *52,* 2154-2158, 2004). Taken together, nowadays data indicate that AMPs presented in RJ could help reduce some of the current crucial problems in beekeeping - the elimination of residues after long terms application of chemotherapeutics. There is an urgent need to development of a method for large scale application of AMPs of bee origin as new, honeybee antibiotics in prevention of honeybee colony against microbial pathogens.

The COLOSS network

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The COLOSS network (prevention of honeybee COlony LOSSes) has been established to explain and to prevent large scale losses of honeybee colonies via identification of the underlying factors and development of emergency measures and sustainable management strategies. For that purpose, international standards will be developed for monitoring and research in form of a BEE BOOK, which will enable large-scale international efforts. COLOSS concerts national activities and comprises of scientists, beekeepers and industry with the aim to create transnational synergies. It currently consists of 203 individual members from 48 countries in four working groups (monitoring and diagnosis, pests and pathogens, environment and beekeeping, genetic diversity and vitality). The networking is facilitated through conferences but more important also through workshops, training schools and short term scientific missions for scientists, extension specialists and apiculturists. This global strategy for prevention of colony losses is therefore based on a broad transnational platform with a focus on the transfer of science into practice and politics. The latter is crucial, especially because socioeconomics and politics have often been neglected as drivers of decline for beekeeping. Only if we succeed to bridge the gaps between bee scientists, apiculture and politics will we achieve sustainable progress in the prevention of colony losses. Therefore, COLOSS will transfer results to all stakeholders responsible for bee health, train extension specialists and enhance public awareness of honeybee health. In conclusion, it seems as if only a global network on bee health will successfully enable collaborative international efforts to limit honeybee decline.

THURSDAY 09:20 – 12:20
Theme V. OPEN SESSIONS: Symposium 15b. Open Session

Varroa control with Flumethrin after 12 years use in Bulgaria

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This study was designed to evaluate the acaricidal effectiveness of Bulgarian Veterinary Medical Product (VMP) Varrostop against Varroa mites in honeybee colonies to establish the Varroa resistance after 12 years use of this product. The treatment of a hundred and thirty-five colonies from 6 apiaries took place in spring and autumn 2009. In our experiments were put down two strips of Varrostop (3.6 mg per 1strip of flumethrin) in every treated hive for 35 days. The number of natural mites drops in the control groups and mites killed by Varrostop (V) treatments during the experiments was estimated by counting the mite drop-down onto the sticky paper sheets at the bottom of the hives. The number of dead mites that had fallen onto the sheets, was used to determine mite mortality in both groups (controls and trials) after control treatment with Perizin (P). The following formula was used to estimate the percentage of mites killed by the treatments: Efficacy = (V/(V + P).100)%.
The infestation rate of Varroa in controls and treated colonies before and after treatment was estimated. Colony strength and behavioral changes in the bees were evaluated visually and both groups – control and treated were compared before and after treatment.
Our result shows high effectiveness of Varrostop – 98,95 (from 98,13% to 99,47%).
These data corresponding to findings of other researchers (Alloui et al., 2002, Gregorc et al., 2007). Flumethrin is administered in hives in a very simple manner, and is non-harmful for the honeybee. By means of utilizing highly effective substance, which is synthetic pyrethroid Varrostop (flumethrin), successful control against Varroa was achieved in our tree treatments and no resistance of Varroa mite was observed after 12 years use.

Contamination of guttated droplets after dressing of seed with neonicotinoid insecticides: a risk to honey bees?

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The dressing of seed is a long known method to protect young growing plants against insect pests. So far, seed dressing with insecticides which are known to be toxic to bees have been classified as a non-toxic application method for honey bees and other non-target insects because the active ingredients are placed into soil. However, the neonicotinoids represent a new generation of insecticides with high xylem mobility within the treated plant. This is an essential prerequisite for the translocation of the active ingredients to peripheral plant tissue, from where they could be released in guttated water. Guttation is a process occurring in plants when leaves can not evaporate water by transpiration when the dew point of the surrounding air is reached. We performed experiments in several agricultural crops in order to demonstrate the release of compounds via guttation in springtime. Fluids were sampled from April to July 2009 with intervals of two days. Compounds were identified and quantified by LC-HR-MS. The results reveal that guttated droplets may contain appreciable quantities of neonicotinoids which could be toxic to water collecting foragers. Released concentration depends on the type of the crop and the age of the plants. However, so far it is still unclear to what extend and in which way the foraging bees use the collected water, particularly how it is distributed within the hive to other bees. For future risk evaluations of pesticides, the possible exposure of bees to systemic insecticides via guttation must, therefore, be considered.

Age and gender specific release of sex pheromone in the honeybee mite Varroa destructor

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The reproductive cycle of the ectoparasitic honeybee mite *Varroa destructor* takes place exclusively within the sealed brood cells. Female mites start oviposition 60 to 70 hours after capping with a haploid male egg followed by up to 5 diploid female eggs in regular 30 hour intervals. The adult males join the faecal accumulation site and wait for freshly moulted females to arrive for mating. In order to analyse the mating behaviour, choice tests with female deutochrysalis, young freshly moulted adult females, and elder female mites were performed using a new bioassay. Our tests confirmed that young females were significantly more attractive than elder mites or deutochrysalis. Obviously, the females become attractive during the process of moulting. Deutochrysalis before moulting were not attractive at all and the attractiveness of the adult female mites decreased with advancing age. By testing of different solvent extracts of young females we could clearly prove that the mating behaviour of the male is elicited by a volatile sex pheromone of young females. This pheromone consists of very few substances of the polar fraction of the extracts. However, also extracts of female deutochrysalis, elder females and even males had an attracting effect. This surprising result indicates that the biological active substances are synthesized in both sexes already during the last nymphal instar. However, only in the freshly moulted female mites the substances are emitted in concentrations sufficient to elicit the mating behaviour of the males. The possible use of these results for a biological control is discussed.

Pollinator communities of Guaiacum sanctum (Zygophylaceae) in two Caribbean islands

Jose Javier Fumero-Caban*, Lourdes Lastra, Elvia J., Melendez-Ackerman

Public communication of honey bee research

Karin Alton*, Norman L. Carreck, Francis W.L. Ratnieks

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Public outreach is an important aspect of the work of the Laboratory of Apiculture and Social Insects (LASI) at the University of Sussex. LASI operates an open door policy whereby its research and expertise are communicated not only at an academic level, but through visits from politicians, farmers, the media, and both corporate and private individuals. Regular talks are given to colleges, beekeeping groups and at networking meetings. We encourage visits from local primary school classes, where learning about bees and the environment has become an integral part of their curriculum. In addition to our public communication drive, we have established a website, www.sussex.ac.uk/lasi which is accessible to all. Information about the research conducted at LASI, under-graduate teaching and pages of resources are available for downloading. The website is also an opportunity to keep the public informed about current news and upcoming events in our lab.
This presentation aims to highlight the importance of the public communication of science, particularly at a time when honey bee research enjoys such a high public profile.

Genetic diversity and evolutionary history of honey bee (*Apis mellifera* L.) populations in Ethiopia

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The honey bees of Ethiopia have long been recognized as peculiar within the African context. Several studies based on morphometric data led to controversial interpretations. Regarding variation of mitochondrial DNA, honey bees from Ethiopia deviated substantially from the known lineages and were grouped in a separate molecular lineage termed Y. To resolve the controversial question of Ethiopian honey bee microtaxonomy and to further characterize the molecular variation of this population, we conducted a comprehensive study on samples from throughout Ethiopia.
Samples from 33 locations were subjected to morphometric, mtDNA and microsatellite analysis, including classified reference samples from adjacent subspecies. In the morphometric analysis, the honey bees of Ethiopia formed a distinct and well-separated group in relation to the honey bees of neighboring areas of Africa, although with a considerable range of morphological variation closely linked to elevation. Most of the observed mitochondrial haplotypes consisted of restriction patterns of lineage Y, which appeared confined to the Ethiopian mountain system. Although a few samples with haplotypes of lineages O and A were observed in the periphery of the sampling area, haplotypes considered typical for East Africa were very rare, indicating restrictions to gene flow between Ethiopian bees and surrounding populations. The results of the microsatellite analysis also supported restricted gene flow between samples inside the volcanic dome system of Ethiopia and adjacent populations in neighbouring areas of Africa.

Genetic variation of honeybee(*Apis mellifera* L.) populations in Iran using RAPD markers

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Honey bees (*Apis mellifera* L.) are the most economically valuable pollinators of agricultural crops worldwide and benefit humankind in exceptionally broad ways. The aim of the present study was to assess the genetic variation and establish the relationship amongst five Iranian honey bee populations from different parts of Iran viz. Tabriz, Khoy, Orumieh, Ardabil and Karaj. A total of 92 unrelated DNA samples were genotyped using 8 RAPD DNA markers. The primers yielded 50 polymorphic bands and numbers of bands were variable from 5-9 (average 6.25) and percentage of polymorphic loci was 78. The estimated genetic diversity ranged from 0.30 (P, % of polymorphic loci =%78) in Karaj population to 0.37 (P =%90) in Ardabil population and total gene diversity among loci was calculated as 0.38 while average within population genetic diversity was 0.3413 and G_{ST} value was 0.0943 among the studied honey bee populations. The genetic distance ranged from 0.0323 (between Khoy and Tabriz) to 0.0804 (between Karaj and Khoy). The dendogram showed two main clusters, the first one included four populations (Tabriz, Khoy, Ardabil, Orumieh) which belongs to North Western of Iran; and the second one included one population (Karaj) only. It can be concluded that in this study RAPD markers could properly grouped the study populations based on their geographical distribution.

Bumble bee cryptic taxa discrimination by pheromonal and morphometrics approach

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The holarctic subgenus Bombus s.str. comprises the most common bumble bee's species. However, the homogenous morphology of the 20 species leads to a problematic systematics, probably the most confused within the genus. Some species are nearly impossible to be identified and several taxa are involved in cryptic species complex. For instance, the lucorum complex includes at least 9 different taxa. The specific status of these cryptic taxa is defined here by sexual pheromone analysis (performed on cephalic secretions). Wing shape geometric morphometrics is used to characterize these taxa by clear morphological variation and, as non-destructive methods, can be applied on museum and reference material (type material). Both methods have been successfully applied on the European species of Bombus s.str. (Bombus cryptarum, B. lucorum, B. magnus, B. sporadicus and B. terrestris) and on two Asian species of the same subgenus as comparison group (B. hypocrita and B. ignitus). Geometric morphometrics of cryptic taxa is based on predictive discriminant analysis. Assignment of potentially independent taxa iss assessed by pattern recognition system. Results of the study show that the close species of the lucorum complex (e.g. B. cryptarum and B. magnus) can be distinguished both by their CLG secretion and by their wing shape providing more evidences about their specific status.

POSTER PRESENTATIONS

Theme I. BEE LOSSES Symposium 1. Pests, Pathogens and bee loss I: focusing on Varroa and viruses

1.P1. Evaluation of honey bee colonies' survival rate according to their different infestation level with Varroa destructor

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Due the lack of any background data and information, concerning seasonal development of *Varroa Destructor* and it consequent influence on the honey bee colonies (*Apis mellifera* L.), three experimental bee yards were established in different regions in Republic of Macedonia. The main idea was to focus on monitoring of *Varroa* mite seasonal development and subsequently assessment of survival rate as a result of different starting infestation levels in October 2009.Each bee yard consisted of 10 colonies which were installed with sister queens from three different lines and with approximately same number of bees. Beside genetic and colony size equalization, there was a different starting infestation level for each of the experimental bee yards. The first bee yard (Skopje) had average infestation level of 0,6 mites in 10 grams of bees. The second bee yard (Bitola) had an average of 2,74 mites in 10 grams of bees and the third bee yard (Probistip) had an average of 16,36 mites in 10 grams. The *Varroa* mite infestation level in each colony was examined by using method of washing 30 grams of bees in October 2009.During the winter season all colonies were inspected every 2 weeks for evaluation of survival rate. In the experimental bee yard (Probistip) with highest starting infestation level (in average 16,36 mites in 10 grams), the first colonies collapsed in January 2010 and the complete bee yard was lost in March 2010. The colonies in remaining two experimental bee yards were still alive during the last inspection in March 2010.

1.P2. Impact of Varroa destructor on hemolymph protein concentration of the worker brood, Apis mellifera intermissa

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In this study, we investigated the impact of *Varroa destructor* on hemolymph protein concentration of the brood of worker, *Apis mellifera intermissa*. Three stages were chosen: Pupa with whith eyes, pupa with brown eyes in the early stage of pigmentation and the pre-emerging stage, provided of northern Algeria.
Our results showed that there was no difference in the protein concentration between parasitized and no parasitized pupa (1-3mite/cell), but the protein concentration was significantly reduced in pre-emerging bees.
Keywords: *Apis mellifera intermissa*, *Varroa destructor*, hemolymph protein, Northern Algeria.

1.P3. Effect of varroa destructor on physiology of worker honey bee, Apis mellifera intermissa and other damage

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In order to evaluate the effect of varroa destructor Anderson and Trueman (Acari: Varroadae) on physiology of worker honeybees (Hymenoptera: Apidae). Two parameters were study: the metabolit response and the immune pathogen effect. Samples of healthy worker honeybees and parasitized nurses provided by the apiary of Oued Aissi (Tizi-Ouzou) located in the North of Algeria were collected. This parasite significantly affected the hemolymph protein. The immune pathogen effect of the Varroa destructor on the nurse honeybees was expressed by a significant reduction (p=0,001) in the total number of hemocytes (THC), which is one of the most frequently used measures for immune system components. This ectoparasitic also affected the length of flagellum antenna, tongue length and the length/breadth of sixth tergite.
Keywords: Apis mellifera intermissa, morphometry, Varroa destructor, hemolymph protein, immune system, hemocytes, Northern Algeria.

1.P4. Serological study of the relation between *Apis mellifera intermissa* and *Varroa destructor*

Haïfa Boudegga*, Med Habib Ben Hamouda

Agronomic High Institute , Chott Mariem. Tunisia

By an immunological study, we put in evidence the presence of specific antigens to *Varroa destructor* in the hemolymph of the three castes of an infested colony: queen, workers and false bumblebees. Antigenic composition of bees' hemolymph is subject to modifications further to an infestation by *Varroa* with the appearance of 4 antigenic constituents only in parasited bees. The multiplicity of these antigens in male heolymph explains well the preference that seems to show the parasite for the male brood which offers a longer duration of development, at the same time allowing a more important number of deutonymphes to finish their development before the emergence of the honeybee male.

1.P5. Chemical Effects of Acrinathrin (Gabon PA92)and 0.1%Copper Gluconate in Control of Varroosis in *Apis mellifera*

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Varroosis is one of the most important disease of honey Bee which is caused by the *varroa. destructor*. This mite destroy Colonies by nutrition via pupa nests. Varroa infest the larva honey bees and living at them and the infested larva produce weak adult honey bees. In Iran several synthetic compounds are used for control of this mite.
In this research, the effectiveness of Acrinathrin (Gabon PA92) and the solution of copper gluconate was compared. 3 experimental groups including group C (control) groupA (Acrinathrin) and group CG (copper gluconate 0.1%) was intended and each group had7 hives.
Before using the drug we took 100 adult bees and their primary infestation to Varroa was determined and 100 pupa cells was investigated from this point of view too,so that if there was not any significant difference among groups so we could start using chemical compounds. this drugs was used as their prescription and 40 days after using, infestation of Bees and larva was measured again. Results was showing the fecreasing in infestation rate from 11.85 to 0.31% in Bees at Acrinathrin group but in CG group was arrised from 11.27 to 20.26% and in control, this level was changed from 13.86 to 25.90%. Infestion rate in pupa at Acrinathrin group had a significant reduction from 12.71 to 1% but in CG and control we have increasing from 13.29 to 30.29% (CG) and 9.86 to 28.86% (C).
Decreasing at Acrinathrin group in each Bee and Larva was significantely ($P<0.05$). the effectiveness rate of Acrinathrin is measured 97.38%. this results are shown that Acrinathrin have a high efficacy but 0.1% copper gluconate solution can not able to control varroosis at the accepted level but it was able to slow down increasing of infestation.

1.P6. Relative virulence of two isolates of Beauveria bassina (BALSAMO) and one isolate of Metarhizium anisopliae against Greater Wax moth (BOISD.)

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Two isolates of *B. bassiana* (AUMC No. 3263 & 3076) and one isolate of *M. anisopliae* (AUMC No. 3085) were tested against the fourth larval instar of the Greater wax moth. In general, mortality rates among treated individuals larvae increased as the conidial concentration increased. Recorded mortality values among the tested concentrations reached 100, 90, 80, 40, 30 and 20% for the concentrations of $5.86x10^5$, $5.86x10^4$, $5.86x10^3$, $5.86x10^2$, 5860 and 2930 conidia / ml for *B. bassiana* isolate No. 3263 and 100, 65, 50, 30, 20 and 10% mortality for *B. bassiana* isolate No. 3076 for concentrations of $5.5x10^6$, $5.5x10^4$, $5.5x10^3$, $5.5x10^2$ and 550 conidia / ml. Recorded mortality values among the treated 4th instar larvae reached 100, 90, 65, 50, 20 and 10% for *M. anisopliae* isolate No. 3085 concentrations of $4.8x10^6$, $4.8x10^5$, $4.8x10^4$, $4.8x10^3$, $4.8x10^2$ conidia / ml, respectively. It seems that the *B. bassiana* isolate AUMC No. 3263 was the most pathogenic for the greater wax moth larvae (739.77 spores / ml) followed by isolate No 3076 LC_{50} = 48292.2 spore / ml, while the least pathogenic isolate was isolate No. 3085 LC_{50} =50156

Theme I. BEE LOSSES: Symposium 2. Pests, Pathogens and bee loss II: focusing on Nosema and viruses

2.P1. The course of Nosema ceranae infection in Poland

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The survey on colony losses done in Poland revealed that during the winter of 2007/2008 these losses reached 15.3%. In 55% of apiaries the probable cause was *Varroa* infestation and in 33% Nosema spp. infection. During the winter of 2008/2009 the losses were significantly lower (about 11.2%), although this time *Nosema* spp. was considered as the main cause (in 60% of apiaries a severe *Nosema* infection was detected). This year the survey is still ongoing, but a rough estimate indicates losses of about 12 %.
In Spain *Nosema ceranae* is considered the main cause of colony losses, though in some countries, such as USA or Sweden, *Nosema ceranae* infection does not seem to be more dangerous for bees than *Nosema apis*. The aim of the ongoing investigation is to observe the course of *Nosema ceranae* infection in Poland
At WULS we are observing experimental colonies which have had pure infection (without *Nosema apis*) of *Nosema ceranae* for at least three years now. A medium level of *Nosema ceranae* infection was found in samples from 2007. We checked the infection in the following years using light microscopy and PCR methods. The results obtained were consistent, *Nosema ceranae* infection was still present in the examined colonies and the colonies are still alive while in Spain *Nosema ceranae* infected colonies seem to die at the end of the second year of infection. Moreover, our colonies swarmed each year, in contrast to the Spanish ones which, despite being very strong, did not swarm in the second year of infection.

2.P2. Evaluation of large-scale dissemination of Nosema ceranae Spores by European Bee- eaters Merops apiaster.

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Identification of transmission routes and of factors affecting the spatial positions of pathogens, hosts and vectors is basic to an adequate disease management. *Nosema ceranae* is a Microsporidian recently described as a parasite of *Apis mellifera* honeybees and is currently considered the aetiological agent of an emergent illness named nosemosis type C. In this communication we report about the likely role of a bird species, the European bee-eater, *Merops apiaster*, as a large-scale dispersive agent of *N. ceranae* by monitoring the occurrence of *N. ceranae* spores in regurgitated pellets of bee-eaters in different areas. We also investigate the role of bee-eater pellets as fomites of *N. apis* and *N. bombi* spores. We found a high prevalence of viable spores of *N. ceranae* in pellets regurgitated by bee-eaters in locations from south Spain to central Asia. In contrast, spores of *N. apis*, considered till recently the most common microsporidium infecting honeybees, were detected in a single locality and *N. bombi* spores were not noticed. Since non-viable spores were also found in bee-eater nests from different locations, this bird species can also reduce the fraction of infected insects by withdrawing pathogens from the colonies. We conclude that bee-eater mobility and migration may have played an important role in the transmission of the pathogen *N. ceranae*.

2.P3. Prevalence of N. apis and N. ceranae in colonies with mixed infection.

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Up to now, two Microsporidia species have been described infecting *Apis mellifera* honeybees: *Nosema apis* and *Nosema ceranae*. Both agents are worldwide spread although during last years *N. ceranae* seems to be the one more prevalent; indeed it has been suggested to be replacing *N. apis*. Denounces of mixed infection in colonies has been observed in different countries, but to date, no data about the prevalence of both agents in a colony has been reported.
In this study we have tested individually 781 bees (400 house bees and 381 foragers) collected from 4 colonies that had been previously identified as infected by both microsporidia. Each colony was sampled four times (October and November 2007 and January and February 2008) collecting 20 foragers and 20 interior bees per sampling.
The microsporidia more prevalent was always *N. ceranae* (29.96%), both in house (20.75%) and foragers (39.63%) bees. On the other hand, *N. apis* was found in a lower level (0.9%) and only in foragers (1.8%). Additionally, bees with mixed infections (*N. apis* and *N. ceranae*) were as well detected although rarely (0.6%) and again only in foragers (1.05%).
Our results suggest that in field conditions *N. ceranae* might have same competitive advantages since its higher presence, compared with *N. apis* in bees from colonies with mixed infection during autumn /winter period.
This work was supported by API-06-009.

2.P4 Variation in honeybee mortality induced by phylogenetically different chalkbrood strains

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Ascosphaera apis is the causative agent of chalkbrood disease in honeybees and is known to reduce brood number and honey production. Recently intracolonial genotypic variation in susceptibility to chalkbrood has been demonstrated, but variation in virulence among phylogenticaly different chalkbrood strains has not been previously tested. After improving the methodology for *in vitro* honeybee brood rearing, we were able to infect larvae from three hives with naturally mated queens. Out of a large collection of *A. apis* strains we selected four strains that came from two distinct phylogenetic clades to infect the honeybee larvae. We found that chalkbrood strain and larval colony origin both affected mortality rates after infection. The two chalkbrood strains from one clade caused relatively low host mortality (12% and 14%), whereas the two strains from the second clade induced much higher larval mortality rates (71% and 92%). In addition, larvae from one of the three hives showed significantly higher susceptibility to chalkbrood infections compared to the other two hives. This study suggests that strain-specific levels of virulence may not be universally applicable across host genotypes. Future research should clarify the degree to which high levels of genetic variation within colony owing to the very high mating frequency of queen honeybees, may affect the epidemiology of chalkbrood infections.

2.P5. A novel method for oral infection of honey bee workers

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To study virulence or other traits of a pathogen a homogeneous oral infection of the individual bee is required. Hitherto existing methods consist of infecting each single bee feeding it manually a defined amount of pathogen in sugar solution (Higes et al. 2006). Here we present a possibility to infect higher amounts of bees individually; assuring that each individual receives the same quantity and is simultaneously prevented to perform food exchange. Freshly emerged workers were placed in 1,5ml tubes individually and these tubes were inserted in a rack and fixed. An opening of about 0.4mm at the end of the tube provided air and the possibility to fed the bees. After 2 hours at RT starving half of the worker were fed with 5 µl of 50% sugar solution containing a standardised amount of the pathogen(s) (e.g. a Nosema spore solution with 2 x 10^5 spores), the rest received pure 50% sugar water. After 30 minutes every bee was released back in cages and worker mortality and infection progress was monitored.

2.P6. Parasitic mite syndrome in colony collapsed apiaries of Turkey

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Honeybee sector characterized by a strong increase in production at the last 20 years making Turkey one of the leading honey producers in the world (Screening report of Turkey for E.U). Unfortunately according to the official reports of government (Legal report register number: B. 12.4. of Dortyol) an alarming number of honey bee colonies began to die in the Hatay province of Turkey since 2005.
Our research group was detected both honeybee viruses and *varroa spp*. within the collapsed colonies of Hatay. This condition has been called as Parasitic Mite Syndrome (PMS) (Shimanuki, 1994). Recently, Turkish beekeeping industry was beset with an unexplained high mortality in colonies infested with *Varroa spp* and infected with viruses (DWV, ABPV, VDV-1, BQCV, CBPV, SBV), bacterias (*Paenibacillus larvae*, *Melissococcus pluton*) and other pathogens (*Ascosphaera apis*, *Nosema ceranea, Nosema apis*).
Clinical symptoms and appearing of *Varroa spp.* may not to enough for identification of viral infections of honeybees. Apiaries without mite infestation and clear clinic symptoms were also found with DWV cases. Detailed epidemiological investigations with molecular tecnics must need to solve the problem of CCD and like syndromes in Turkey.

2.P7. Distribution of Nosema sp. in Spain

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Nosema apis and *Nosema ceranae* are intracellular parasites infecting the midgut of adult honey bees. In recent years, these microsporidian have been associated with CCD and it has been suggested that *Nosema sp* induces higher mortality in honey bees.
In CERA (Apicultural Reference Center in Andalusia) 609 samples from provinces of Spain have been analysed using PCR with capillary electrophoresis.
The presence of *Nosema sp.* has been detected in some provinces in 100% of the samples indicating that this parasite is widespread throughout the country.
The monitoring of sampled hives indicates that the hives are still alive and have normal biological development and production. This means that the presence of *Nosema cerana* and *Nosema apis* is not the reason of the collapse and death of beehives.

Theme I. BEE LOSSES Symposium 3. Monitoring, Diagnostics, and Pathogens

3.P1. Preliminary results of APENET monitoring for bee diseases in Italy

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APENET is a network for studying bees-environment interactions and monitoring honeybee mortality and colony losses in Italy. Here we report the results of the first year (2009) of APENET's activity. 316 representative samples of adult honeybees from 16 out of 20 Italian regions were submitted to the Istituto Zooprofilattico Sperimentale delle Venezie for diagnosis of *Nosema apis*/*Nosema ceranae* infection and for virus detection. Honey bee crushings were examined by light microscopy for the presence of *Nosema* spp spores and then were used for DNA extraction. PCR was performed for a specific 16S rRNA gene region of *Nosema* spp (Higes *et al*, 2006) and, for species identification, the PCR products were sequenced and similarity analysis was performed by using BLAST. For virus detection, honeybee samples were submitted to National Bee Unit, The Food and Environment Research Agency, Sand Hutton, York (UK).
Nosema ceranae was present in all monitored Italian regions, while *Nosema apis* or *Nosema apis*/*Nosema ceranae* coinfection were not detected. Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood Virus (SBV), Acute Bee Paralysis Virus (ABPV) and Chronic Bee Paralysis Virus (CBPV) were detected in Italian apiaries in different combinations per region. Israeli Acute Bee Paralysis Virus (IAPV), Apis Iridescent Virus (AIV) and Kasmir Bee Virus (KBV) were not detected.
APENET preliminary results provide evidence of the enzootic presence of *Nosema ceranae* and for the first time investigate systematically the viruses presence and their geographic distribution in Italian apiaries.

3.P2. Comparison of proteome between infected and uninfected honey bees with the Chronic Bee Paralysis Virus

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The Chronic bee paralysis virus (CBPV) is the etiologic agent of an infectious and contagious disease of adult honey bees (*Apis mellifera* L.) called Paralysis. The genome of this virus is composed of two previously characterized RNAs, RNA1 (3674 nt) and RNA2 (2305 nt). RNA1 and RNA2 respectively encode three and four open reading frames (ORFs). The amino acid sequence of ORF3 on RNA1 has shown similarities with the RNA-dependent RNA polymerase (RdRp) of positive single-stranded RNA viruses whereas the ORF3 on RNA2 encodes a putative structural protein. Although the complete sequences of RNA1 and RNA2 have been determined, CBPV cannot be assigned to any viral family. Consequently, characterization of viral proteins is essential in order to suggest a taxonomic position of CBPV. Indeed, this virus could be the first type specie of a new viral family.
In this study, proteins extracted from hemolymph collected from virus-infected and uninfected bees were separated by two-dimensional gel electrophoresis to identify viral proteins. Differentially expressed protein spots were excised, and after trypsin digestion the peptide fragments were analyzed by matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. Resulting peptide mass fingerprints were compared with those in protein databases and some viral protein sequences have been identified. In addition of these identifications, these results provide first information on differential honey bee protein expression after CBPV infection.

3.P3. PCR-based method to detect the different bacteria associated with the European Foulbrood.

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Honeybee brood was mainly affected by two bacterial diseases, the American Foulbrood (AFB) and the European Foulbrood (EFB). AFB and EFB are respectively caused by one etiological agent, *Paenibacillus larvae* and *Melissococcus plutonius*. In addition, some secondary invaders or associative agents (as *Paenibacillus alvei*, *Enterococcus faecalis*, *Brevibacillus laterosporus*, *Achromobacter eurydice*) could be involved in EFB. However, little information is available on the exact role of these associative agents. Over the past few years, an increase of EFB infected apiaries was reported and some of these cases were considered as highly virulent. The exact reasons for this increase are not completely understood. To assess the possible implication of secondary invaders in these cases, PCR-based method was developed to discriminate the several bacteria involved in this disease. The two bacteria *M. plutonius* and *P. alvei* were identified with previously described PCR. For the three others, specific primers of the *E. faecalis*, *B. laterosporus*, *A. eurydice* taxon were designed and the PCR products sequenced to validate the detection. Different samples of EFB collected between 2004 and 2010 were analyzed and frequencies of the different bacteria were reported. These results could provide important information to understand the involvement of the different bacteria in the multiplicity of EFB cases observed.

3.P4. Sampling details for detecting and quantifying Nosema spp infection levels in honey bee colonies

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For detection of Nosema presence OIE manual prescribes analysis of 60 bees collected from hive entrance. For quantification of infection levels however the same manual prescribes analysis of 10 "older" worker bees.
Due to the growing concern that *Nosema ceranae* may be strongly involved in the colony loss phenomenon, routine analysis of presence of this pathogen in honey bee colonies is becoming more widespread, even in countries where Nosema disease was traditionally not a problem. It is therefore important to establish standard sampling and analysis protocols which couple repeatability and ease of field sampling, and which, together with observations on colony development and health status may provide the basis for the determination of Nosema disease threshold levels. These levels could represent a useful prophylactic tool for beekeepers and limit dissemination of the disease. Here we present comparisons between different kinds of sampling modes and sample sizes, and an overview of Nosema disease in several apiaries in Italy, with observations of seasonal differences.

3.P5. Real-time PCR method for the quantification of *Paenibacillus larvae* spores in debris: comparison to microbiology

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American foulbrood is the most diffused and severe bacterial disease of the honeybee brood. It is caused by the spore-forming bacterium *Paenibacillus larvae* (*P. larvae*). The control of the disease relies essentially on the identification and elimination of diseased colonies. Disclosure of latent infections in colonies without symptoms, by detection of *P. larvae* spores, is an important component of American foulbrood prevention. Honey, adult bees and winter debris are the most useful targets to assess the level of contamination of colonies. The aim of our work was the development of a real-time PCR method for the quantitative assessment of the spore content in winter debris and the comparison of this molecular method to the microbiological enumeration**.** A new TaqMan® real-time PCR was designed targeting the 16S-ribosomal RNA gene of *P. larvae,* taking particular care of reaction specificity. For this purpose both *in-silico* and *in-vitro* specificity were tested with respect to several potential bacterial contaminants of the hive, belonging to *Paenibacillus, Bacillus* and *Enterococcus* genera. The same real-time PCR was used to screen for positive samples of debris and to quantify the spores in samples resulted positive on screening. Microbiological spore counting was performed by plating onto MYPGP-agar supplemented with nalidixic and pipemidic acid, after distilled-water extraction of samples. Winter debris from approximately 150 colonies belonging to 15 apiaries of three provinces in Northern Italy were analysed. Selected apiaries had shown different disease levels in the year preceding the study. The concordance level of the tested methods is discussed.

3.P6. Monitoring of honey bee health and mortality in 5 natural parks in Italy

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The one year-long project to monitor honeybee health and mortality, promoted by the Ministry of the Environment (Ministero dell´Ambiente e della Tutela del Territorio e del Mare) of Italy, co-ordinated by Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) has began on May 2009.
Aim of this project is to superviseinvestigate the effects of the man-made pollutants released in the environment (i.e. heavy metals, pesticides) on the honeybees' health and their products, within 5 Italian natural areas (Parco Nazionale delle Dolomiti Bellunesi, Parco dei Gessi bolognesi e dei Calanchi dell'Abbadessa, Parco di Migliarino San Rossore Massaciuccoli, Riserva Naturale Statale del Litorale Romano, Parco Regionale dei Monti Simbruini).
In the project are involved: the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZS-LT), the Istituto Zooprofilattico Sperimentale delle Venezie (IZS-VE), the

Department of Physiological Sciences of Pisa University and the DiSTA of University of Bologna. Within each natural park, have been identified areas exposed to pollutants and areas not exposed in which have been settled apiaries each one constituted of 20 hives.
Each investigated apiary, have been monitored for the following independent variables: the main species of plants, the prevalent agricultural activities and the measures used for crop protection in the surrounding areas, as well as the man-made pollution sources. Moreover, the colonies have been monitored for health status, worker weekly mortality and amount of brood. Finally, both honey and worker bees have been periodically sampled in order to search for both heavy metals and pesticides.

3.P7. Honey bee virus infections in Croatian apiaries

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In certain cases, e.g. when environmental and beekeeping factors result in stress, honeybee viruses may cause clinical symptoms or even lethal disease in bee colonies. Extensive traffic of bees, trade, exchange of bee queens and introduction of new colonies into apiaries may facilitate the spread of viruses. This work describes the first molecular evidence for viruses in Croatian honeybee samples.
A survey on the occurrence of seven different pathogenic honeybee viruses (ABPV, SBV, BQCV, DWV, KBV, CBPV and IABPV) was carried out using reverse transcription PCR analysis. Samples were collected in 82 apiaries located in 20 different districts of the country, and approx. 100 bees were collected in each apiary.
Results showed that 10.97% of the investigated samples were infected with ABPV, 40.24% with SBV, 29.26% with BQCV, 95.12% with DWV and 9.75% with CBPV. No samples tested positive for KBV and IABPV. Most of the samples were infected with more than one virus. The highest percentage of infection was found for DWV which occurred in 78 out of 82 investigated apiaries.
When comparing the prevalence of the seven most important honeybee viruses detected in Croatia with the results from other European countries, our results revealed that the prevalence of BQCV, ABPV, SBV and CBPV was generally lower, while the occurrence of DWV was equally high than in other countries (>90%). Import of honeybee colonies and bee queens from countries of the European Union to Croatia is rare due to strict regulations on animal health. The comparatively lower prevalence of the above-mentioned 4 viruses might be a consequence of this policy.

3.P8. *Nosema ceranae* infections of honeybee colonies in Croatia

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Nosema disease is a parasitic disease of adult honeybees caused by two species of microsporidia, *Nosema apis* and *Nosema ceranae*. Last one is recently described parasite of European honeybee (*Apis mellifera*) and its geographical distribution is not well known. Disease may have some negative effects on honeybee colonies and cause high losses in economy of apiculture and generally agriculture. The aim of this research was to investigate and determinate the presence of *N. ceranae* and distribution in all 21 district of Croatia. A total of 204 samples of dead bees from different localities were selected and investigated using light microscopic examination and multiplex PCR.
Results showed that *N. ceranae* was only nosema species found to infect honeybees from our widespread collection in all three climatic areas, i. e. medirerranean, mountain and continental part of Croatia. Eleven honeybee samples were negative for both Nosema species, and mixed infections were not detected at all. The nucleotide sequences of amplification products from Nosema infected samples were 100% identical with the *N. ceranae* sequence deposited in the GenBank database.
European and Croatian regulations prohibit the use of antibiotics and fumagillin in the treatment of apian disease because of the potentional development of resistance and concerns about residues. Herbal treatment alternatives are being investigated and recent results with Nozevit demonstrated high effectiveness as a preventive measure and curative treatment for bees infected with *N. ceranae*.

3.P9. Probiotic Lactobacilli in the prevention of *Paenibacillus larvae* infection in Honeybes

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American foulbrood (AFB) is a very dangerous disease that every year affects many honeybee colonies in the world. In most of the countries, based on veterinary legislation the positive bee colonies are eradicated with high financial losses. However, the highest financial loss consists in an indirect loss through decrease of plants pollination. Medicaments application is little effective. Mainly obscuration of clinical symptoms and a risk of accumulation of residues of medicaments in honeybee products occur.
American Foulbrood is a contagious disease that is mandatory to be reported. Sporulating Gr^+ bacterium *Paenibacillus larvae* causes American Foulbrood. Larvae that have died on American foulbrood disease exhibit a ropy condition and can be drawnout to a long thread that may snap back if pulled too far. In this stage we can find spores only.
In the world, the American foulbrood is fought radically or by application of antibiotics and sulphonamides. Administration of antibiotics and/or other factors exposing bees to stress may affect the intestine micro-flora balance.
In the EC, during 2003 and at the beginning of 2008, the State Veterinary Administrations banned introduction of 203 samples of honey to the EU because residues of tylosin, oxytetracycline, chloramfenicol, streptomycin, nitrofuran and sulphonamides were detected in honey samples.
The main aims of this study were - at first to evaluate physiological composition of gut microflora in collected honey bees and to isolate from this microflora lactobacilli, at last to

select strains of lactobacilli for potential probiotic use for the prevention of *Paenibacillus larvae*- the causal agent of American Foulbrood disease.
For the evaluation of microflora composition in healthy adult honey bees collected in May 1999 were used intestines, stomachs and rectal sacks. Gut microflora was compound of total aerobes (10^6 cfu.g^{-1}), total anaerobes (10^7 cfu.g^{-1}), lactic acid bacteria ($2x10^5$ cfu.g^{-1}), *Lactobacillus* sp. ($1x10^3$ cfu.g^{-1}), *Enterobacteriaceae* ($2x10^5$ cfu.g^{-1}), *E. coli* ($4x10^5$ cfu.g^{-1}). *Enterobacteriaceae* were identified as *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *E. coli*, *Providentia rettgeri*, *Proteus vulgaris*, *Enterobacter cloacae*, *Enterobacter aglomerans*, *Serrtia sp.*, *Citrobacter sp.*. Furthermore *Bacillus megaterium*, *Bac. subtilis*, *Bac. cereus*, *Staph. epidermidis*, *Staph. aureus*, *Staph. hominis*, *Staph. saprophyticus*, *Enterococcus faecalis*, *Bifidobacterium* sp. and *Lactobacillus* sp. were characterized. These results are in accordance with findings of other authors.
Selected lactobacilli were tested for their autoaggregation properties, ability of long-term storage surviving at -20°C, antibacterial activity against *Penibacillus larvae*, and growth properties. There were selected 40 strains of lactobacilli. Strains were stored in freezing glycerine medium at -20°C, 20strains for 5 months and 20 strains for 5 years. 55 % resp. 60 % of strains survived 5 month resp. 5 years storage. All strains which survived storage showed autoaggregation abilities in MRS broth. Inhibition of *Paenibacillus larvae* was tested by paper disc assay. Based on diameter of inhibition zone, tested strains were divided into the non-inhibiting, weak to middle inhibiting and strong inhibiting strains. From 17 tested strains only 2 strains did not inhibit and 3 strains inhibited strong the growth of *Paenibacillus larvae*. Two of these 3 strains were identified as *Lactobacillus brevis* and the third one as *Lactobacillus plantarum*.

3.P10. Susceptibility of *V. destructor* to synthetic pyrethroids after many years of their application in polish apiaries

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In Poland, the following three compounds have been longest used in the fight against *V. destructor*: amitraz, fluvalinate and flumethrin. Currently only the first two: amitraz (Apiwarol - tablets for burning) and flumethrin (Bayvarol strips) are registered, but fluvalinate (homemade strips) is nonetheless still very often used by beekeepers. Purpose of this study was to determine whether the application of fluvalinate only for several successive years resulted in a reduction in the susceptibility of *V. destructor* to flumethrin.
For laboratory testing Varroa mites were collected from bee colonies treated with fluvalinate for at least five years and from colonies treated with other substances (organic acids, amitraz). The testing procedure was similar to that used by Milani (1995). The Varroa mites were placed for 6 hours into the Petri dishes with different flumethrin concentrations (0.5; 1.0; 2.0; 5.0; 10.0; 50.0 and 100.0 µg/g). Number of living and dead parasites was evaluated after 6, 24 and 48 hours. Estimated average values of flumethrin concentration LC50 and LC95 for both parasites groups did not differ significantly. The value of LC50 for *V. destructor* population from bee colonies treated with fluvalinate amounted to 0.3µg/g and for parasites from the apiaries treated with other substances was 1.2 µg/g. Value of LC 95 reached 41.7 µg/g and 26.9 µg/g respectively.

3.P11. Molecular tools for detection of replicative forms of SBV and BQCV in *Vespa velutina*

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In 2004, the presence of the hornet *Vespa velutina* L. was observed for the first time in France accidentally introduced probably via garden pots imported from China and becoming a major threat for the honeybees. The aim of this study was to evaluate the presence of honeybee viruses in Asian hornets and to develop molecular tools for the detection of their replication. The presence of CBPV, ABPV, IAPV, DWV, SBV and BQCV was studied by RT-qPCR (CBPV) or RT-PCR (others viruses), on adult and larvae collected in 2008 in the South West region of France. CBPV was weakly detected in few samples. Larvae of Asian hornets were detected positive for SBV, BQCV and DWV, while adult were detected positive for DWV and positive or weakly positive for BQCV and SBV. To evaluate the ability of SBV and BQCV to replicate in this host, we developed a RT-PCR specific to the detection of the minus-strand RNA of these viruses. First strand cDNA was synthesized using a minus-strand specific primer, consisting of a tag unrelated to the viral sequences coupled to a primer specific of the researched virus. A minus-strand-specific PCR was then performed using a forward primer corresponding to the tag sequence with a SBV or BQCV-specific reverse primer. Moreover, PCR products were sequenced to check their specificity.
Replication of SBV and BQCV was thus demonstrated by detection of the minus-strand RNAs in larvae. These results show that the *Vespa velutina* L. might represent a natural reservoir for bee viruses.

3.P12. First Report of *Aethina tumida* in Europe

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The Competent Authorities reported the first (and only) diagnostic of *Aethina tumida* (Murray) larvae in honeybees Apis mellifera ligustica in Europe Portugal, in 2004.
The larvae of *Aethina tumida* were observed on "candy" which fulfilled the boxes where the queen where transported
Texas, United States of America in October 2004.

3.P13. Prevalence of *Acarapis woodi* in honey bee (*Apis mellifera*) colonies determined by one-step PCR

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In this study we describe a new one-step PCR method for diagnosing tracheal mite (*Acarapis woodi*) infestation in honey bees. Molecular methods previously described to detect *A. woodi* required two rounds of PCR, increasing the risk of contamination. Besides, new sequences of the *Acarapis* genus (*A. externus* and *A. dorsalis*) have been recently published in GenBank and this can undermine the usefulness of old PCR designs since the primers previously described for detecting *A. woodi* can also anneal in these other species, losing the specificity of the method.
Using phylogenetic tools, we demonstrate that the patterns of genetic differentiation across the *A. externus* and *A. dorsalis* sequences raise serious concerns about the current species classification of these organisms. Our method is based only on *A. woodi* sequences and it is fast, accurate, sensitive and can be carried out by laboratories that lack updated or expensive equipment. The short size of our amplicons allows the detection of *A.woodi* even if the samples are not perfectly preserved (degraded DNA). In order to assure the reliability of our technique, we have sequenced all the positive samples, resulting *A. woodi* in all the cases.
We have also determined the real prevalence of *A. woodi* in the honey bee colonies in professional apiaries from Spain in the years 2006 and 2007 by studying 1967 samples randomly selected (house worker bees). Traditionally, this pathogen has not been taken into consideration due to the lack of the typical symptoms in the bee colonies and to the unavailability of accurate detecting methods. Nevertheless, we have found that A. woodi is present in Spain at considerable levels, reaching a prevalence of 12,7% (CI95% = 9,8 - 15,6) in spring and 19,5% (CI95% = 15,1 - 23,9) in autumn 2007.

3.P14. Risk factors associated to the honey bee colony losses in a high-honey-producter area of Spain.

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Epidemiological risk factors for Honey Bee Colony losses were investigated in a cross-sectional study in the north-western Spanish region of Galicia, a geographical area really affected for this phenomenon in the last years. A representative population from the area was surveyed during spring 2008. Information relative to different risk factors was collected from laboratory testing the samples and from an epidemiological-questionnaire. Case definition was established as: the disappearance of adult bees from the hives with no or little build up of dead bees in or in

front of the colony; and significant autumn/winter mortality in the honey bee colonies with no prior symptoms. Laboratory test included the diagnosis in adult/coomb bees of following pathogens: *V.destructor*, *N.ceranae, N.apis, Acarapis spp*, *P.larvae*, *M.plutonius, A. apis*, BQCV, KBV, AIV, IAPV, SBV, DWV, ABPV, CBPV and the presence of the main agrotoxic, insectides and acaricides residues in stored pollen and palinological analysis of pollen. Univariant analysis followed by 3 types of regression appropriated for cross-sectional studies were employed to detect relationships between the colony losses and potential risk factors. Spatial correlations were explored using Local Getis-Ord Gi*(d). In the final models only *N. ceranae* was significant and strongly associated to the colony loss. The prevalence was 53.5% ($CI_{95\%}$=3.2-63.9;p<0.0001). Other variables detected in the univariant-analysis should be explored in further researches. No clusters were identified.

3.P15. Spring prevalence of different viruses in honey bee colony in Spain

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The presence of Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV) and Sacbrood Bee Virus (SBV) has been determined in a cross-sectional study including the most of Spanish territories.
In the present work, 284 adult honey bee samples collected from different geographic regions of Spain during spring 2006 have been analyzed for the presence of the RNA viruses BQCV, DWV and SBV by Real Time PCR using specific primers for each virus and SYBR Green. Samples were macerated in AL buffer + Carrier (Qiagen) and supernatant selected for RNA extraction. RNA integrity was assured by previous test of β-Actin.
Viral infection rates resulted low, being 12 %, 12 % and 1 % for BQCV, DWV and SBV respectively. Analysis of other viruses is currently being carried out.
This work was supported by RTA2005-00152 (FEDER FOUNDS).

3.P16. Prevalence of *Paenibacillus larvae, Melissococcus plutonius* and *Ascosphaera apis* in Spain.

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The incidence of *Paenibacillus larvae, Melissococcus plutonius* and *Ascosphaera apis*, the causative agents of American Foulbrood, European Foulbrood and Chalkbrood, respectively, has been studied in a cross-sectional study including the most of Spanish territories, by means of a multiplex PCR.
A total of 1659 brood samples randomly distributed all over Spain and collected in spring and autumn 2006 and 2007 were checked. In addition, 85 apiaries randomly selected were analyzed for presence of these infectious agents both in adult honeybee and brood. All the samples were crushed independently and DNA was extracted (*BioSprint 96 DNA kit*).
Our diagnostic method enables a rapid and specific detection and identification of *P. larvae, M. plutonius* and *A. apis* in only one step from infected bee larvae or adult honeybees. The prevalence average of these pathogens was usually low. *A. apis* (2.57%) was the one more frequently found, and *M. plutonius* was rarely found (0.3%). Finally, *P. larvae* had an intermediate prevalence (1.54%). No geographical pattern was found in their distribution
The prevalence of *M. plutonius* and *P. larvae* was 2-fold higher in adult bees than in brood. For *A. apis* was very similar in both (17,7% in brood *vs.* 15.3% in adult bees). These data support the role that adult bees can play in the dissemination of infectious agents within and/or among bee colonies, which could be source of infection for brood, causing disease under some appropriate conditions.
Work supported by RTA2005-00152 (FEDER-FOUNDS).

3.P.17. Detection and genetic typing of acute bee paralysis virus in Slovenia

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Acute bee paralysis virus (ABPV) is a honeybee pathogen widespread as a latent and inapparent infection. The virus spreads by way of the salivary gland secretions of adult bees and in food stores to which these secretions are added. ABPV has been identified as a major factor contributing to the mortality of honeybees in colonies infested with *Varroa* mite.
86 samples of death worker carniolan honeybee (*Apis mellifera carnica*) were collected from 67 different locations (apiaries) in all parts of Slovenia. Samples were collected from January 2007 to March 2010 and most of them were associated to mortally episodes or sudden bee family collapse, which were in high number observed in this period.
For the detection of ABPV one step RT-PCR protocol was used. A specific RT-PCR product (398 bp) of ABPV was detected in 39,5 % (34/86) of examined samples. Sequencing of 381 bp long segment of 11 amplified RT-PCR products in both directions was performed and multiple alignments were created with Muscle program. The first Slovene ABPV nucleotide sequences were clustered into 2 distinct genetic groups. Ten sequences were clustered in the same genetic group which share 98-100 % homology to each other and 95 % nucleotide homology with the closest sequences of isolates from Poland, Germany and Austria (AY053366). In second genetic group we detected only one Slovenian isolate with the highest homology (96 %) to Hungarian isolates (AY053377).

3.P18. Contribution on fungal contamination of honey

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Some samples of five kinds of Polish honey (lime, poly-floral, honey-dew, nectar-honeydew honey) were collected from private, stationary apiaries localized in Puławy region (middle-south Poland).They were suspected of yeast infection because of their organoleptic features, i.e. sour taste, disagreeable smelland unpleasant looks because of its characteristic irregular crystallization; there was every indication on fermentation process in this product. The aim of this study was identification of fungal microorganisms infesting feral samples of honey. Collection and organoleptic analyses of samples were made in Department of Bee Products, Apiculture Division in Pulawy, Research Institute of Pomology and Floriculture in Skierniewice; isolation and identification of fungi isolated from honey were made in Departrment of General Biology, Medical University in Bialystok (Poland). Experimental methods was described in earlier publication (Kiziewicz et al. 2008).
Microbiological analyses of samples and microscopic identification of isolated fungi show that honey was infested with following species of these microorganisms: *Aspergillus flavus* Link, *Candida albicans* (C.P. Robin), *Geotrichum candidum* Link, *Penicilium chrysogenum* (Thom) (= *P. notatum* Thom), *Saccharomyces (Zygosaccharomyces*) *rouxii* (Boutroux).
All kinds of honey were infested with yeast, *G. candidum* and some with *S. rouxii,* causing fermentation of honey. Mould fungi (*A. flavus* , *C. albicans , P. chrysogenum*) cause moulding and spoiling process of infested products. They are also producers of strong allergens causing allergy diseases (bronchial asthma/bronchitis, conjunctivitis, rhinitis, skin inflammation/dermatitis) and mycotoxins harmful for health.
Results of introductory investigations seem to be interesting and well-founded; they will be continued in future.

4.P1. Heterologous expression of honeybee calcium channel subunits, a new tool to study sensitivity to insecticides

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Voltage-gated sodium and calcium channels play key roles in neuronal and muscular excitability and are known targets for a number of insecticides. Data obtained recently on honeybee olfactory antennal neurons showed for instance dramatic effects of pyrethroids on bee sodium channels (Kadala et al. 2008). We demonstrated that honeybee muscle calcium channels are sensitive to pyrethroids as well (Collet 2009). However, to date, high throughput screening molecular tools needed to study the role of sodium and calcium channels in the physiology and toxicology of the honeybee are still lacking.

With respect to voltage-gated calcium channels our groups have identified in the genomic honeybee database three pore-forming CaVa subunits that could belong to the CaV1, CaV2 and CaV3 families, a single CaVb and one CaVa2d subunits. Cloning of these genes revealed unique amino-acids sequences that may confer to these channels specific biophysical and pharmacological profiles. This is confirmed by a preliminary characterization of the auxiliary calcium channel b subunit (AmCaVb) expressed in oocytes with mammalian CaVa subunits. As expected, AmCaVb increases current amplitudes and modifies the voltage-dependent gating parameters of the expressed current. AmCaVb also modifies the kinetics of the currents in a more specific way. In situ hybridizations and immuno-histochemistry with specific antibodies will determine the expression pattern of calcium channels in honeybees and will precise their potential roles in cellular excitability. These results may help in understanding the deleterious effects of currently used insecticides or in constructing new tools for the screening of more selective insecticides.

4.P2. Evaluation of bee brood development using automated digital image processing and analysis

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Adverse effects of plant protection products on development of the honeybee (*Apis mellifera*) brood under conditions close to the open environment are assessed according to the OECD guidance document 75. This evaluation requires assessment and correlation of the developmental stages in a large number of cells in several treatments and replicates at several time points. Manual counting of such systems is time consuming, of limited reproducibility and difficult to verify. These deficits can be resolved by purpose-built computerised image analysis tools.

We optimized computer-assisted digital image acquisition to deliver high resolution images with maximal contrast and minimal reflections. For the analysis of the images, customized macros of the freeware program “ImageJ” were created, providing automatic identification of the combs and an optimal interface to assess the developmental stage of the larvae. The features include:

1) jumping from comb to comb at optimal magnification,
2) non-destructive user-defined labelling of the staging of individual eggs, larvae or pupae,

3) galleries of the same comb on consecutive images of the same frame at different time points,
4) the compilation of results for data evaluation.
Ongoing development is aimed to implement pattern recognition of the combs' content, allowing the completely automated assessment of entire bee brood studies in a batch mode and also enabling the measurement of additional morphometric parameter (e.g. size, shape, staging).
The digital evaluation significantly accelerates the assessment of honeybee brood studies and allows full documentation of these studies with possibility of control and revision at the individual comb level.

4.P3. Insecticidal maize seed coatings and honeybees - precautionary measures and experience from Austria 2009 and 2010

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The Austrian Federal Office of Food Safety set compulsory precautionary measures to be followed by seed plants and farmers concerning insecticidal maize and oil pumpkin seed coatings in order to reduce the exposure risk of honey bees. Most important elements are: appropriate coating and labelling of the seed, use only against *Diabrotica virgifera* and wireworm (larvae of elateridae), adaption (deflector) of pneumatic corn sowing machines, careful handling of coated seed, minding direction and velocity of wind while sowing to avoid contamination of blooming off-crop areas.
In 2009 suspected bee poisoning incidents connected with corn sowing were reported from 28 apiaries. In these cases no total colony losses occurred, but losses of forager bees, house bees and bee brood and in some cases reduction of honey yield were observed. Samples of the affected apiaries were tested for residues of Clothianidin, Thiamethoxam, Imidacloprid, Fipronil and Fipronilsulfon. 83 % (n=29) of the bee samples and 64 % (n=36) of the bee bread samples showed positive results for at least one of the analytes. No residues were found in extracted spring honey samples (n=8). All plant samples (n=14) collected from off-crop areas near affected apiaries were tested positive for residues. Bee samples of the affected apiaries were analysed for parasites and pathogens, just in some cases they showed low infestation. As results indicate, the observed symptoms were linked with the period of maize sowing and the use of insecticidal maize seed coating.
Experience of the ongoing season 2010 will be reported.

4.P4. Screening on 200 commonly used pesticides in agriculture in Andalusia (South of Spain)

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Pesticides used in agriculture can have devastating effects on bees. Effects of fipronil (phenylpyrazole) and imidacloprid (neonicotinoid) are very known but there are other molecules affecting honey bee biology.
A screening of 200 commonly used pesticides in agriculture has been carried out in Andalusia on samples of soil, sunflower, corn and bees by means of the methodology 165 GC-MS and 35 HPLC.The pesticides detected and the concentrations obtained have been the next: Malation 130 µg/Kg, Oxifluorfen 210 µg/Kg, Trifularina 310 µg/Kg, DDT 20 µg/Kg, DDE 30 µg/Kg, Diuron 20 µg/Kg and Endrin 30 µg/Kg were detected on samples of Soil. Metalaxil 10 µg/Kg was

detected on samples of Plants. Azinfos etil 30 µg/Kg and Clorpirifos etil 190 µg/Kg were detected on samples of Honeybees.
These results indicate other substances in the environment may have a direct or indirect impact on health honey bees.

4.P5. Direct powdering with neonicotinoids of foraging bees avoiding agriculture drilling machine.

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In the last few years honeybees colonies all over the world has been interested by an intense death phenomena, this syndrome has been called Colony Collapse Disorder (CCD) (Underwood et al. 2007, vanEngelsdorp et al. 2009). In Italian central areas maize is cultivated intensively (more than 1 million hectars) and in the period of corn sowing has been observed the sudden disappearance of many worker bees and also the presence of many bees death in front of hives. Those phenomena has been associated to the use of insecticides for coating the corn seeds and the use of pneumatic drilling machines; in 2002 it's been discovered how drilling machines emits in atmosphere particulates of coat (Greatti et al. 2002) that can reach herbaceous vegetation that grow nearby fields. Another contamination way that was studied is the collection of drops emitted by young corn plants containing a.i. used for the coat (Girolami et al. 2009). A new contamination way has been explored through this study by accustoming worker bees to visit a food dispenser that was achievable by flying over a field where a pneumatic drilling machine was working. Honeybees where captured on the dispenser at established time from the beginning of sowing, put inside cages and observed the intoxication symptoms until 48 hours from capture. High mortality percentage was observed on bees captured during experimentation, demonstrating how honeybees get poisoned by direct contact with the insecticide particulates originated from the drilling machine while they are avoiding it during foraging flights.

4.P6. Effects of acaricides on varroa control and biological characteristics of honeybee *Apis mellifera intermissa*

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The effectiveness of four acaricides (Bayvarol, Apivar, Apiguard and ApiLife Var) against *Varroa destructor* was evaluated with use of infested colonies of *Apis mellifera intermissa*, housed in Langstroth standard hives. Per acaricide, five hives were treated, and one group of five hives was left untreated as the control. All acaricides significantly reduced the levels of varroa mite infestation on adult honeybees and worker brood, but the efficacy was higher for Apiguard (93-97%) and ApiLife Var (94-98%) compared to Bayvarol (85-90%) and Apivar (82-88%). We determine eventual secondary effects of these treatments on the metabolism of the bees by measuring the amounts of protein, carbohydrates and lipids in the hemolymph and body tissues and acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) activities in the adult stages of *A.m.intermissa* as biological endpoint of secondary pesticide effects. Five groups (acaricide treatments and control) of three hives each were used. The treatment is done from the egg to the adult stage in order to sample adult workers which have been exposed to acaricides during their whole development. The results showed that acaricides affect honeybees. The two thymol formulations ApiLife Var and Apiguard impaired less the amounts

of protein, carbohydrates and lipids of the honeybees than Apivar and Bayvarol. All acaricides have no significant effect on AChE activity. However, they led to increase GST activity in the emerged and nurse bees, as compared to controls. In the forager bees, the GSTs activity was similar in all groups of honeybees. Bees are exposed to toxic stress when acaricides, especially synthetic ones, are used as treatments in hives. Overall, the data indicated that essential oils like Apiguard and ApiLife Var can be recommended in the control of *V. destructor* and beekeepers should take into consideration of timing and concentration when using acaricides.

4.P7. A survey of pesticide residues in stored pollen from honey bee colonies (*Apis mellifera*) in a Mediterranean country

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In the last decade, an increase in honeybee colony losses has been reported in several countries. The causes of this decline are still not clear. This study was set out to evaluate the pesticide residues in stored pollen from honey bee colonies and their possible impact on honey bee losses in Spain. A total of 469 representative apiaries were randomly selected.
All pollen samples were subjected to a multi-residue analysis to determine residues of herbicides, organophosphorous and other insecticides (including neonicotinoids), organochlorine compounds, acaricides, polycyclic aromatic hydrocarbons, and a palynological analysis to confirm the type of foraging crop.
Pesticide residues were detected in 38% of samples collected in spring, and only in 24% of samples collected in autumn.
Fluvalinate and chlorfenvinphos were the most prevalent pesticide residues detected in the samples analyzed.
Fipronil was detected in 7% of all the spring samples and never in autumn samples. More than 47.8% of stored pollen samples belong to wild vegetation and pollen from sunflower were only detected in 10.4% of the samples.
Further studies will be necessary to determine the possible role of the more frequently detected pesticides (such as acaricides), and their synergism with other pathogens more prevalent in our country.

4.P8. Mitochondrial DNA variation in populations of Algerian honey bee *Apis mellifera sahariensis* and *Apis mellifera intermissa*

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The diversity of the bee, its natural range, is suffering the effects of human practices in general and agriculture in particular (inappropriate use of pesticides or mass). The inappropriate use of pesticides results in a sharp decline in population, would be likely to deplete the gene pool of a large number of species including the bee Saharan Africa was nearly decimated by the locust control campaigns in 1965 , 1987 and 2003.

Molecular analysis of populations of native bees (Apis mellifera and Apis mellifera sahariensis intermissa) based on PCR amplification of the intergenic region between the genes for subunits I and II of cytochrome oxidase, shows that bees belong Algerian the African evolutionary lineage (A). Four haplotypes were identified (A1, A2, A8 and A9). The distribution of these haplotypes found by region shows that the Algerian haplotype A9 and A8 are abundant haplotype in almost all regions of Algeria. However, the haplotype A9 is more abundant in the regions of El Bayadh (100%), Medea (100%), Oran (67%), Ain Sefra (63%) and Bechar (55%). For other regions (Algiers, Bejaia, Batna, Mostaganem, Skikda, and Tlemcen Tebassa), the frequency is 33%. Similarly, the haplotype A8 is more abundant in the following regions: Tiaret and Saida (100%), Algiers, Boumerdes, Mostaganem, Skikda and Tebessa (67%) and Batna, Oran, Setif, Sidi Belaabbes, Bechar (33%).

Keys words : *Apis mellifera sahariensis* and *Apis mellifera intermissa* , mtDNA, molecular analysis

4.P9. Relationships between honey bee health and the use of neonicotinoid seed dressing in maize.

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The use of neonicotinoids in seed dressing is considered one of the main causes of recent bee losses. This research aimed to investigate the factors affecting honey bee health in areas with maize plants grown from neonicotinoid dressed seeds.
The conditions of colonies, located in these areas for experimental purposes, were evaluated in the aftermath of sowing, to avoid possible direct effects on bees due to the active ingredients from the powders dispersed by mechanical drills.
15 hives divided into three groups were set in three areas in the surroundings of Turin. Station 1 hives were located adjacent to maize fields planted with Gaucho dressed seeds, while station 2 hives were located in a small town surrounded by maize fields from Poncho and Gaucho dressed seeds. Station 3 was located far from maize crops.
The assessment was carried out on a weekly basis on the following factors: the health status and the ongoing development of the families, the mortality of bees through "underbasket" traps, the fall down of varroa (naturally occurring and after specific treatments), and the number of dead bees with deformed wings. The possible collection by bees of drops during guttation and of pollen during flowering were also examined.
Data were analysed using the GLIMMIX procedure in SAS.
The three stations colonies did not show significant differences in strength and mortality.

4.P10. A survey of pesticide residues in pollen collected by honey bees in Slovenia

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Pollen is a traditional beekeeping product used by man as a component of dietetic food and a source of biological active substances. Beside carbohydrates, proteins, fibre, lipids, wide spectrum of different substances such as vitamins, minerals and trace elements, many other residues could also be present in the pollen. In 2009, 90 honey bee (*Apis mellifera carnica*) colonies from 30 different locations were monitored from four different types of agricultural practice (1) intensive field production 10, (2) apple orchards 6, (3) vineyards) 4, (4) agriculturally extensive locations 10; to investigate the quantity of residues in the pollen from different locations in Slovenia. Altogether 50 pollen samples were collected and analyzed. Analyses using LC/MS/MS (Liquid chromatography-mass spectrometry) and analyses using GC/MS (Gas chromatography/mass spectrometry) with DRS (Deconvolution reporting software) were performed to search for residues of 880 different (chemicals) (including pesticides) in the pollen. Residues of pesticides were found on 11 locations (37 %). Altogether residues of 16 compounds were found in samples. The content of residues ranged from 76 to 0.011 mg/kg. The majority of them were fungicides (69 %). All investigated samples from wine growing locations were contaminated with residues of pesticides, 67 % of samples from fruit growing locations and 40 % of samples from field production. One sample on the agriculturally extensive location was contaminated with one insecticide (chlorpyriphos-ethyl).

Theme II. DIVERSITY AND CONSERVATION Symposium 5. Diversity of bees

5.P1. Interactions amongst the genetic origin of the bees, the environment and pathogens in Poland

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In the beekeeping season of 2009 three experimental apiaries were created in Poland. The apiaries were located in various parts of the country with different climatic and foraging conditions. Altogether 124 colonies with bee queens belonging to 8 lines were prepared. Afterwards, groups of colonies with bee queens of 4 different lines were situated in each apiary: south-east part of the country, Kunki - 37 colonies: CarG GR1 from Pulawy, Poland, CarP Kortowka from Olsztyn, Poland, MacB Macedonica from Bulgaria, Mel P Augustowska from Poland; central Poland, Bronowice near Pulawy - 44 colonies: CarC from Croatia, CarG GR1 from Pulawy, Poland, CarP Kortowka from Olsztyn, Poland, Car V Veitshöchheim from Germany; and in northern Poland, Gasiory - 43 colonies: CarC from Croatia, CarK from Kirchhain, Germany, CarL from Lunz, Austria, CarP Kortowka from Olsztyn, Poland.
All the colonies were of similar strength before wintering with 13.309 of bees on average (from 11.854 to 15.456). In October, different amounts of brood were observed in the colonies. The highest number of cells with brood was noted in colonies in northern Poland (20.302) compared to central and south-east (respectively 1654 and 1589). In autumn, all experimental colonies were treated against *Varroa* mite with Apiwarol AS with amitraz in the form of fumigation and with oxalic acid. The highest number of mites fallen on hive board after treatments was observed in apiaries in Kunki and Gasiory (respectively 4.5 and 3.5 mite per 100 bees) and the lowest in Bronowice (1.1 mite per 100 bees). In some of the colonies *N.apis* or *N.ceranae* was found, while in very few colonies - both, *N.apis* and *N.ceranae*. Number of colonies lost during winter 2009/2010, recorded in mid-April was: in Kunki-15, in Bronowice-9 and in Gasiory-2.

5.P2. Wintering bee colonies composed of workers of different genetic origin

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Poland is the largest producer of inseminated bee queens in the world. Artificial insemination was initially used only in controlled selection in breeding apiaries but within recent years, tens of thousands of inseminated commercial queens have been produced. Selection of parents is usually individual. Queens are inseminated with semen collected from drones from one father colony. In nature, however, queens mate with drones from many colonies what results in a high genetic diversity in a colony. Low genetic diversity contributes to lower both vitality and survival of bees. Wintering abilities of bee colonies was examined in Puławy – Poland. Two groups of colonies with Carniolan queens were used: queens inseminated with mixed semen collected from drones from 30 colonies that belonged to three breeding lines (MS-Mixed semen) and ones inseminated with semen collected from the same colonies, but the difference is that every queen was inseminated with semen from a single colony (SCS-Single colony semen).
The colonies of the SCS group wintered on average of 5.3 combs of danant type hive while the ones of the MS group on average of 4.8 MS combs. During the spring inspection the number of combs was adjusted to the colony strength. Significantly more combs were taken from colonies of SCS than from ones of MS group, respectively 0.68 and 0.48.

Autumn infestation of bees with *Varroa* destructor was smaller in the colonies of MS -216 mites per colony compared with the others' 269 mites per colony, but not significant statistically. Average winter debris of bees was also lower in colonies of MS-967 bees per colony than SCS-1154 bees per colony, also not significant statistically. The infestation of bees with *Nosema* spp. in the winter debris was examined. A similar number of colonies free of protozoa; low, medium and heavily infested with *Nosema* spp. was observed. Losses of colonies or losses of queens were also similar in both groups of colonies. There was no correlation stated between *Nosema* spp. infestation and losses of colonies. However, the correlation coefficient between the autumn bee infestation with V. destructor and the strength of colonies in the spring was r = 0.5. Research financed by the Ministry of Science and Higher Education, grant number 527/N-COST/2009/0.

5.P3. Similarity of Polish lines of bees based on the morphological features

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There were 45 registered breeding lines of bees in 2008 and they belonged to three races: *Apis mellifera carnica*-33, *A. m. caucasica*-8, *A. m. mellifera*-4. Only 15% of them originated from Polish geographical area, other ones were imported. Large-scale import of bees began in the 60's. Samples of bees for morphometric measurements were taken from colonies of all breeding lines. Following characteristics were measured: the length of the proboscis, the width and the fourth tergit and cubital index (body & wing features) as well as the coordinates of the junctions of veins on the wing and the size of the wing (wing features). Results of these measurements were subjected to the cluster analysis with Ward's method of agglomeration rule. Bee lines were then grouped in accordance with similarity of morphometric characteristics. The first analysis was based on body & wing features (3 variables), the other one used wing features (39 variables). Both hierarchical tree diagrams showed three different groups according to the bee race, declared by the breeder. In hierarchical tree diagram based on three variables, only one bee line did not fit its race group. Euclidean distances were low within the lines of native Black and lines of Carniolan bees and also for several breeding lines of one bee race kept in individual breeding apiary. Hierarchical cluster tree based on wing features showed that 6 bee lines did not fit the group of declared race. It can be explained by the fact that the only cubital veins (two measured distances) of the wing had been used in the selection and the cubital index is not always correlated to the remaining points on the wing, which are characteristic for given bee race.

5.P4. Molecular analysis discriminated among Serbian ecotypes of *Apis* mellifera carnica

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To determine the genetic architecture of honeybees from Serbia, 37 colonies were collected from four regions (Banat, Timok, Syenichko-Pershterski and South-east) and 24 reference colonies from Albania, Bosnia and Herzegovina, Italy, Macedonia and Croatia were included. Two subspecies of *Apis mellifera* inhabit the territory of Serbia, *A. m. carnica* and *A. m. macedonica*, and both subspecies belong to the eastern Mediterranean (C) evolutionary lineage based on morphometric and molecular analyses. The genetic structure and molecular

diversity of Serbian honeybee populations have been analyzed in this study with 12 *loci* microsatellites and mitochondrial markers in order to discriminate between Serbian honeybees ecotypes. All the detected haplotypes belonged to the C evolutionary lineage. The Bayesian analysis of the microsatellite variation in the Serbian honeybee population differentiated three ecotypes (Banat, Timok and Syenichko-Pershterski) previously defined with morphometric, chromosomal and behavioural analysis. Further assignment test revealed that the honeybee ecotypes from Serbia could belong to *carnica* and *macedonica* subspecies as also the honeybees from Bosnia and Herzegovina and Albania. The molecular data suggest that the distribution range of *A. m. macedonica* is larger than currently known and also the area of hybridization between both subspecies. The description of ecotypes in Serbian honeybees and the hybridization areas must be considered in future conservation strategies.

5.P5. Genetic analyses of Algerian honey bee populations and relationships with other honey bee subspecies.

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The honeybee (*Apis mellifera* L.) is an ecologically and economically important insect species. Two subspecies of 26 identified subspecies are distributed in Algeria. The importation of foreign queens and migratory beekeeping practices are factors that can affect the genetic diversity of the Algerian honeybees. In this way, it is necessary to first accurate knowledge about the genetic structure of the populations. The aim of this research is to study the genetic structure of the Algerian populations of honeybees, to examine their phylogenetic relationships and to investigate the possibility of the gene flow existence as a result of migratory beekeeping and commercial breeding. The genetic diversity was investigated using fourteen polymorphic microsatellite loci. Eight different populations of 438 colonies were analysed and the microsatellite analysis showed that the honeybee populations are characterized by a higher level of genetic variation in terms of average number of alleles and degree of heterozygosity and the majority of the populations are at Hardy-Weinberg equilibrium. Phylogenetic and population structure analyses support clustering of these populations in one principal group, confirm that Algerian honeybees are belonging to the lineage A and are completely separated from the other lineages M, C and O. The microsatellite genetic homogeneity within Algerian populations indicates that the structure of the local populations is not affected by the modern beekeeping practices.

5.P6. Temporal patterns of honey bee (*Apis mellifera* L.) mitochondrial DNA variation in the archipelago of Azores (Portugal)

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Morphological and molecular studies have been carried out on different Mediterranean and Atlantic island populations of honey bees. A previous genetic survey of the Azorean honey bees, carried out by De la Rúa and colleagues (2006), showed their genetic distinctiveness from continental populations and their close relationship with NW African populations. Herein

we present the results of a more comprehensive survey (samples collected from all the islands of the archipelago) of the mitochondrial DNA variation exhibited by the honey bee populations of Azores. Using previously obtained results from honey bee samples collected in 2001, we assess the temporal maternal variation of these populations over a 9 year time frame.

5.P7. Spatial patterns of honey bee (*Apis mellifera* L.) genetic diversity in continental Portugal: the story told by mitochondrial DNA

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Over 24 honey bee (*Apis mellifera* L.) subspecies occur naturally in Europe, Africa and the Middle East. Morphological and molecular markers have grouped this wide-ranging diversity into four lineages (A, M, C, O). The Iberian Peninsula harbours two of such lineages (A and M) and the greatest honey bee maternal diversity and complexity across Europe. While the Spanish honey bee populations have been extensively surveyed for mtDNA variation, the genetic composition of the populations inhabiting the Portuguese side of the Iberian Peninsula is virtually unknown. Herein, we present the first comprehensive account of the maternal variation across continental Portugal. Over 1000 colonies were surveyed for the COI-COII mitochondrial DNA region, which showed a high genetic diversity across Portugal, mostly haplotypes of African origin (lineage A).

5.P8. Honeybee (*Apis mellifera* L.) in Turkey: Biodiversity using Geometric Morphometrics Analysis.

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The wing shape morphology of honey bee population of Turkey was examined by geometric morphometric analysis using the coordinates of 18 landmarks located at vein intersections of the right wing. After obtaining the wings images, the vein junctions were detected automatically using DrawWing software. Generalized Procrustes Analysis and Principal Component Analysis were used to compare the shape of venation. The analysis on wing shapes revealed significant information on population differentiation. Projections into the first two canonical plane, slightly separated honeybee populations in three main groups, as well as UPGMA dendrogram: Southeastern Anatolia and Thrace and the other groups inclueding the remaining populations. Honey bees of Southeastern Anatolia remained as a distinct unit and showed different pattern in terms of shape morphometry among honeybee populations of Turkey. Also the ANOVA analysis of the log of centroid size of wings showed differences amonge all honey bee populations. Geometric morphometrics method can be a very powerful tool in exploring intra-specific variation at the population level and evolutionary studies concerning honey bees in Turkey.

5.P9. Variability of some production traits of selected lines in Republic of Serbia

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Improving population honey bee *Apis mellifera carnica* Poll. Serbia is of interest to the wider region of Southeast Europe. Breeding and selection of honey bees in Serbia carried out by the system of line breeding. Each line is examined a dozen of daughter queens that come from prominent founders of the queen mother. The paper presents results of the production characteristics of four isolated lines in the area of western Serbia. The amount of bees, brood, honey and pollen was investigated in two spring and one autumn view. All bee colony is located in a standard Langstroth Root hives and grade examinations carried out each frame surface at 1/10.
Area of bees in the first spring inspection varied from 2.15 frame in the line D to 2.95 frame in the line G, while in autumn exam the largest surface of bees had lines Z and G (2.35 and 2.28 frames).The best spring development measured as difference in the amount of brood between the two exams had a line K (1.94 frames). Honey surface in the spring and fall in line G was for 5,98% and for 13.71% higher compared with the general average of the tested lines. Also, the G line had 22.8% larger area of pollen in the spring compared to the general average of lines. All tested lines exhibited variability, which provides enough space for their further improvement and separation the best queen mothers.

5.P10. DNA barcoding reveals genetic variation within three neotropical stingless bees species (genus *Scaptotrigona*)

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DNA barcoding has been applied in many animal taxa and it is now advocated as a reliable and rapid means for species-level identification. Due to this, it has been chosen to analyze the inter and intra-specific variation in three stingless bees species from Central America (Mexico and Guatemala): *Scaptotrigona mexicana, S. pectoralis* and *S. hellwegeri.* Phylogenetic analyses of the DNA barcoding region have shown molecular differences among the populations of each species in coincidence with their geographical origin
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5.P11. **The bumblebees of the Italian western Alps**

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The western Alps extend from Bocchetta di Altare to the Splügenpass. They belong to France and Switzerland on the external side and to Italy on the internal one. The boundary runs mostly on the watershed between the Po basin and the Rhone and Rhine basins, except for the upper Ticino valley that belongs to Switzerland.
Bumblebee faunas of France and Switzerland are much better known than the Italian one. That is especially true for the Italian western Alps, where only the "Waldensian Valleys", a small area in the Turin district, were thoroughly investigated in the years 1946-1970; information on the remaining area is rather old and scanty. Therefore, in the late nineties we started a survey of bumblebees of the Italian western Alps in order to ascertain actual species distribution and, whenever possible, the changes occurred in the last decades due to anthropogenic disturbance, habitat alteration, and global warming. Until now, several localities were sampled throughout the area, but our efforts were mainly focused on the Susa valley in the years 2000-2006 and on the Aosta valley in 2009.
Available data, both from the literature and our investigations, were subdivided according to the administrative districts and grouped, in each district, on a chronological basis (until 1970, 1971-1995, 1996-today). Information appears concentrated mostly in the region Valle d'Aosta and in the province of Turin, which are located centrally in the western Alps, and rarefies moving on either side with the remarkable exception of the Canton Ticino in Switzerland.

5.P12. Influence of habitat type on distributions of bees in Afyonkarahisar province, Turkey

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Afyonkarahisar is located in the meeting point of the Middle Anatolia, Aegean and Mediterranean regions of Turkey. It contains many different habitats such as high mountains, a natural parks, swamps and lakes. Besides, there are huge agricultural (arable) areas found in this region. However the pollinator bee fauna of this province haven't been exposed so far. Therefore, a four-year survey was conducted in this region between 2006 and 2009 to determine the faunistic properties of this area. In the first step of the research the taxonomical identifications were made on the 5152 collected specimens. As a result, 163 species belonging to Apoidea (Hymenoptera) superfamily were identified. Secondly, and more significantly, the distributions of this species according to different habitat types were analyzed. The GPS (Global Positioning System) coordinates and other recorded data of the collected material were used to perform GIS (Geographic Information System) database. This database was combined with the Corine Land Cover Classification (CLCC) data of Afyonkarahisar. According to three different habitat types (arable, forest and pasture) and five different elevations, the distributions of the species were evaluated. As a result, the species richness of arable areas were found higher (N: 137) than forest (46) and pasture areas (25). It was also found that the majority of the species (N: 127) were found in the lowest altitudes (811-1000m). The findings suggest that the lower elevations and the arable areas which comprise the vegetation of a mixture of cultivated crops and natural flowers provide good habitats for bees.

Theme II. DIVERSITY AND CONSERVATION Symposium 6. Drivers of bee loss in Europe and impacts for society

6.P1. Forecasting shifts in ecosystem services under scenarios of climate change: The case of honey bees and honey production

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Honey bees provide invaluable ecosystem services that range from the production of honey and wax, to the pollination of plants in natural and managed ecosystems. Given the strong dependence of honeybee activity on climate, we focused on the island of Puerto Rico and asked (1) What is the relationship between honey yield and climate? and (2) What is the range of variability in honey yields that we might expect under several scenarios of climate change? To answer these questions we used honey yields and climatic variables to develop an ensemble of models that were used in a GIS to examine the spatial variability in honey yields under current and future climate change scenarios. Honey yields were obtained from bi-annual surveys conducted by the Department of Agriculture of Puerto Rico and the climatic variables were derived in BIOCLIM from monthly average maximum and minimum temperatures and total monthly precipitation for 1998-2005. Historical and contemporary (1910-2005) records indicate that honey yield averages (5.3L/colony) have changed little over time but that honey yield variability has decreased due to a reduction in maximum yields. The different models predicted minimum and maximum honey yields at 0.6 and 67.0 L/colony for the current scenario, and at 0.00 and 31.3 L/colony for the climate-change scenarios. The models, in general, coincide in the predicted location of above-average honey yields (>5.3 L/colony), but differed in the aerial extent of these areas, from 1007 km^2 (Model 1) to 2257 km^2 (Model 4). These results illustrate the possible impacts of climate change on honey production and ultimately the services provided by honeybees.
Keywords: *Apis mellifera*, honey production, forecasting models, climate change, BIOCLIM, ecosystem services

6.P2. Unmanaged honey bee colonies in the UK

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It is widely assumed that, particularly because of current disease threats, feral or unmanaged honeybee colonies will not survive in W-Europe without beekeeper intervention; and that if unmanaged colonies are found they are likely to be swarms from the current year that will quickly perish. Anecdotally however, this does not seem to be the case with reports of honeybee hives persisting in old buildings or trees for many years. My project aims to shed light on the status of these unmanaged honeybee colonies in UK landscapes. To that end I will:

- Assess survival of unmanaged colonies: Genotyping will be used to compare the queen lines over time. Daughter queens created by supersedure can be distinguished from local swarms.
- Examine the predominant race of unmanaged colonies that survive: Genoptyping will be used to assess race and levels of introgression from other races. It has been suggested that the native race *Apis mellifera mellifera* should be better adapted to the UK's environmental conditions and have a higher rate of survival. Paired comparison of unmanaged and managed honeybee colonies will explore the geneflow and patrilines to examine the genetic relationship between colonies.

Compare disease levels between managed and unmanaged colonies using quantitative PCR: I will assess levels of deformed wing virus, black queen cell virus, sacbrood virus, chronic paralysis virus, acute bee paralysis virus, Kashmir bee virus, Israeli acute paralysis virus, cloudy wing virus*, Nosema ceranae, Nosema apis,* American foul brood and European foul brood in paired managed and unmanaged colonies.

6.P3. Mass bee colony losses in Poland - study on environmental factors and pathogenic organisms in 2010

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Since 2009 we have started a research project "Definition of the role of environmental, genetic and pathogenic factors in mass bee colony losses"as a part of the COST ACTION FA0803: PREVENTION OF HONEYBEE COLONY LOSSES (COLOSS) founded by Ministry of Science and Higher Education.
The purpose of this project among other studies is also to assess the presence and role of environmental and pathogenic factors in the phenomenon of mass bee colony losses in Polish apiaries. In last winter 2009-2010, bee colony losses were higher in comparison with 2008/2009 winter season. From March to May 2010 we have collected materials to the laboratory studies from 250 apiaries where phenomenon of mass losses of bee colonies was observed. In these apiaries, total colonies were sampled
Apiaries were classified as:

- having no colonies with CCD symptoms and losses of bee colonies were above 10%
- having colonies with CCD symptoms and losses of bee colonies were above 10%
- losses of bee colonies were up to 10 % (control)

The study involves:

- determination of prevalence of several pathogens (*V. destructor*, *Nosema* spp. viruses: ABPV, CBPV, IAPV, DWV) in the samples of adult bees and bee brood
- analyses of pesticide residues used in agriculture in the beebread, sugar stores and adult bees (GC/MS method)
- analyses of miticide residues (used for control *Varroa destructor*) in brood nest wax and comb foundation (GC/MS method)
- analyses of adulteration with hydrocarbons of the brood nest wax and comb foundation (GC/MS method)

analyses of interactions between these risk factors and their synergistic effects on the increased bee colony mortality

6.P4. Competitive Interactions Between Honeybees and Wild Pollinators

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It is widely documented that managed and wild pollinators and the services they provide are under increasing threat from different sources. One of the most important threats suggested to affect wild pollinators is competition with invasive or managed honey bees (*Apis mellifera*). Not much is known, however, about the extent or mechanism of interaction. Competitive interactions between honey bees and wild pollinators are considered among the most important mechanisms driving community structure. Yet the extent to which honey bees alter wild pollinators and consequently the pollination services they provide remains controversial. I experimentally tested the effects of competition by honeybees on wild bee foraging behaviour, and the potential impact this may have on the functioning of pollination and seed set.

Theme III. BEE BIOLOGY and ECOLOGY Symposium 7. Bees and pollination

7.P1. Bumblebee (*Bombus terrestris* L. 1758) pollination of cherry (*Prunus avium* L.) fields in Afyonkarahisar and Ankara, Turkey

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Bumblebees (*Bombus spp*.) are well known important pollinators for both the pollination of wild flowers and pollination services in outdoor, greenhouse horticulture and orchards. In commercial use, the foraging range determines the optimal density of bumblebee colonies for facilitating pollination services. The results of the previous studies that focus on the foraging of the bumblebees are range from a few hundred meters to several kilometers. The foraging distance is depended on several factors like climate change, weather conditions and also the plant density.
In this study, bumblebee activity was investigated in two different orchards located in Ankara and Afyonkarahisar provinces. Both orchards were described with its size, shape and also with the number of plants, varieties of trees, plant ratios, age of the trees, planting structure and surrounding vegetation. *Bombus terrestris* colonies were settled in the fields. Foraging activities of the workers were determined by transect observations, pollen collection and counting the flower visits. The temperature and relative humidity were also recorded in the blooming period of the cherries.

7.P2. Foraging preferences of bumblebees in a lowland in northeastern Italy

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Bumblebees (Hymenoptera, Apidae) are wild social bees; like honeybees, they gather nectar and pollen to feed their offspring. Their presence is normally associated with flower rich grasslands since they require a continuous supply of food throughout the flight season. Unfortunately a dramatic decline of bumblebee species was reported throughout Europe as well as in northeastern Italy. The aim of this research was to assess foraging preferences of bumblebees in meadows located in a lowland in northeastern Italy.
From May to September 2007-2009, field observations were carried out in two meadows differing for plant species composition as regards in particular the percentage of synantropic species. Bumblebees on flowers were counted and identified and competition plants, flowering in the same period, were noted.
In the more natural meadow the most abundant species was *Bombus pascuorum*, while in the other site, characterized by many synantropic plants*, B. lapidarius* and *B. terrestris* were more represented. Longer-tongued bumblebeee species, like *B. pascuorum*, were strongly related to flowers with long corollae like *Betonica officinalis*; instead, shorter-tongued species, like *B. terrestris*, seemed to prefer short corollae flowers like *Centaurea* spp.. Both the abundance and species richness of flowering plants and bumblebees were positively correlated. Some plants, like *Clinopodium vulgare*, were visited by pollinators only for nectar, others as *Filipendula vulgaris* for pollen, while *Centaurea scabiosa* for both rewards. The knowledge of plants, utilized by bumblebees as nectar and pollen sources, represents a first step in planning actions to restore their populations and to prevent further declines.

7.P3. Potential Apoidea pollinators of the bean *Vicia faba* L. (Fabaceae) in Algiers region

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Bees have a key role in the pollination of plants and crops, including agriculture. Every purpose of the study, these were conducted throughout the year 2009 to define and identify the most important species of bees deployed on the ground some flowering crops and estimate the numerical density and study its behavior in flower pollination at the test station of the National Institute for Agriculture at El Harrach, Algiers. Results showed the presence of three different species of wild bees of the level of membrane wings (Hymenoptera) are *Andrena, Eucera* and *Xylocopa. Eucera* found the sex of the broad beans for the duration of its flowers and the months of April, May. The genus *Andrena* and *Xylocopa* found on herbal plants after the flowers period of beans. Every means that the two species have not a special type of vegetation compared to the specie *Eucera*. And the results also indicate that there was a difference in the behavior of bees and the activity of the three genus that have a significant role in crop pollination beans.

7.P4. Pollination requirements of apricot: ten years of research in Piedmont (Italy)

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Apricot (*Prunus armeniaca* L.) is usually autocompatible, while lately some autoincompatible cultivars have spread because of their quality excellence. The introduction of new cultivars needs therefore the evaluation, besides of agronomic and productive requirements, of also the necessity of insect pollination so to adopt adequate planting and control strategies compatible with the accomplishment of the pollination service
Observation and test were carried out on 22 newly established cultivars in three localities of the Cuneo district (north-western Italy): CReSO Experimental Stations of Cuneo (2000-2004) and Manta (from 2008 onwards) and the fruit-growing farm Quaranta of Costigliole di Saluzzo in 2005. In any case there was plenty of plants supplying compatible pollen and beehives were placed close to the experimental orchards in order to grant an adequate insect pollination. During the blooming period observations were made on the presence of wild pollinators, that were however rather scanty.
For each cultivar and each year 3-5 trees were selected. On each tree 2 fruit-bearing branches of similar size were chosen; one of them was isolated with a net mesh sufficient to prevent the passage of pollinating insects, without hampering sensibly wind action, while the other one was left free. For each branch flowers, set fruits and ripe fruits were counted; the latter were also weighed.
The cultivars that produced no or very few fruits on the caged branches are autoincompatible and require therefore the presence of pollinating cultivars, combined with the beneficial action of the honey bee.

7.P5. Bumblebees are key pollinators of a critically endangered fritillary (*Fritillaria meleagris* L., Liliaceae)

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Fritillary (*Fritillaria meleagris* L., Liliaceae) is a critically endangered Polish red-list plant. It is regarded as protogynous and xenogamous species pollinated by *Bombus terrestris* bumblebees. However the data on the plant's biology is rather fragmentary and mostly based on 19th century observations. For four seasons we studied pollination biology of *F. meleagris* in the largest Polish population in SE Poland and found that its flowers are visited by 14 insect species from two orders: Hymenoptera and Diptera. Although the largest *F. meleagris* pollen loads may be carried by solitary bees (*Andrena, Lassioglossum*), based on video records of insect behavior and analyses of their pollen loads we found that the most efficient pollinators include four species of bumblebees: *B. terrestris, B. lapidarius, B. hortorum* and *B. sylvarum*.

7.P6. Pollination network in a lowland meadow

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Mutualistic relationships among flowering plants and insect pollinators play a crucial role in generating and maintaining biodiversity in terrestrial ecosystems. Some authors consider them as "biodiversity architecture". Such relationships usually link dozens or even hundreds of species forming complex mutualistic networks. Their structure is very diverse and asymmetric – most of taxa are rather weakly linked to the others while a few are more strongly connected to the rest of species than could be expected from random distribution of links in similar network. The paper presents the structure of a large pollination network in a wet lowland *Molinietalia* meadow in NE Poland.

7.P7. Effects of transgenic pear pollen on honey bees

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Honey bees are the most important pollinators of fruit trees and are a key test species used in the biosafety assessment of genetically modified crops. To assess potential impacts of transgenic pear trees for honey bee colonies, adult honey bees were fed with a sucrose solution containing non-transgenic pear pollen or pollen, expressing antibiotic resistance genes nptII and hpt. Transgenic pollen also contained marker genes gus and gus-intron, respectively. We evaluated weight, longevity and flight activity of worker bees, queen productivity, sealed brood, and productivity of bee colonies. Studies showed no negative effects of transgenic pollen on these parameters. Moreover hpt pollen-fed bees did differ significantly from control bees in the timing of their longevity, flight activity, sealed brood, and honey production, whereas nptII pollen-fed bees - flight activity and honey production only. Our results suggest that the transgenic pear has no adverse impacts on honey bees. To our knowledge, this is the first report of testing transgenic pollen of fruit trees on honey bees.

8.P1. Analysis of nuclear copies of mitochondrial sequences in two sister species of *Melipona* (Hymenoptera: Meliponini)

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Transposed copies of mitochondrial DNA into the nuclear genome (numts) have been frequently reported in many hymenopterans species, including a high density in the honeybee nuclear genome. The accidental amplification of numts in phylogenetic studies focused on mtDNA highlights the importance of a correct determination of numts and their related mtDNA sequences. We report here the discovery of a numt derived from a mitochondrial 16S gene in the stingless bees species *Melipona colimana* and *M. fasciata*. PCR products were cloned in both species obtaining thirty orthologous numts and specific primers were also developed. Numts were identified by the presence of deletions and insertions and the disruption of the 16S rDNA secondary structure.

8.P2. Acute oral toxicity of Thiamethoxam on the honeybee *Apis mellifera sahariensis* and *Apis mellifera intermissa.*

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The use of plant protection products in the fight against crop pests is a necessity. But this control method is not without risk and may result in unintended consequences that are manifested by toxicity in non-target organisms like beneficial insects. Among them, honeybees play a triple role, agronomic, economic and ecological.
The commercial preparation Actara 25 WG containing the Thiamethoxam is recognized as being toxic to bees and it is prohibited for use during the period of flowering. However, this product is systemic, it is therefore present in low concentrations in the treated plant throughout its development cycle. What are the effects induced in the bee by contamination by low doses of Thiamethoxam that might be contained in the pollen and nectar when flowering, that is the question?
To try to answer this problem, we determined in a first step the sensitivity of the honeybee Saharan and Tellian *Apis mellifera sahariensis* and *Apis mellifera intermissa* testing acute oral toxicity of thiamethoxam on bees in the laboratory which constitutes the basis of pattern toxicological risk assessment. The study is based on the determination of LD 50 oral. Bees of unknown age were fed with sucrose solutions with increasing doses of the insecticide (1, 10, 20, 50, 70, 90, 100ng of active substance per bee). Throughout the duration of the study treatment and control bees were placed in the dark (T ° 25 ° C ± 2 ° C and 60% RH). The result showed that the LD50 obtained are around 13.08, 6.78 and 6.60 ng / bee *Apis mellifera sahariensis* and order of 12.50, 13.52 and 10.54 ng / bee *Apis mellifera intermissa* respectively 24h, 48h and 72h after treatment.
Keywords: Thiamethoxam, *Apis mellifera intermissa, Apis mellifera ahariensis,* LD50, Acute toxicity,

Theme III. BEE BIOLOGY and ECOLOGY Symposium 10. Learning and memory in honey bees

10.P1. DNA recombination and repair-related mechanisms and aversive learning and memory in honey bees.

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The honey bee, Apis mellifera, has been used as a model system for neuroethological and behavioral studies including navigation, social organization, learning, and memory. Some have attributed memory formation to transcriptional/translational events. Other studies, have reported DNA recombination and repair as important mechanisms in memory formation. Recently, the recombination factor Flap structure-specific endonuclease 1(fen-1) has been shown to be required for memory formation of conditioning taste aversion in rats. Since memory formation requires the regulation of gene expression, DNA recombination and repair mechanisms may be directly involved in the process. Studies on honey bees have been extensively documented, but only a few have focus on aversive learning. In the present study we tested aversive associative learning in forager bees with a novel protocol. In brief, bees were trained to associate a specific context to the aversive stimulus, a mild electric shock. A short-term memory (STM) test was performed shortly after training to determine the ability of the bee to avoid the environment where it previously received the noxious stimulus. Next, brain dissections and mRNA extraction were performed and followed by fen-1 mRNA qRT-PCR on individuals who showed learning in the STM test. Our results shows thavvt the fen-1 gene expression was significantly induced in the brain of trained bees when compared to controls in this type of avoidance shock training, thus suggesting that the fen-1 gene expression is involved in aversive memory formation in honey bees.

Theme III. BEE BIOLOGY and ECOLOGY Symposium 11. Nutrition and physiology in bees

11.P1. Parameters influencing load weight and drinking time of water foraging honeybees

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A honeybee colony needs water to regulate the hive temperature on hot days by evaporative cooling, to dilute stored honey, and for the consumption by nurse bees to produce jelly for feeding of the larval brood. Water is collected during the whole breeding season by specialized foragers. We measured the body temperature, the load weight and the duration of stays of water collecting bees on a rain-water barrel.
The duration of the foraging stays declined exponentially from 113 seconds to 27 seconds as ambient temperature (T_a) increased from 5 to 38 °C. The mean crop loading increased linearly from 48.7 to 61.7 mg water as the T_a increased from 11.5 to 25.0 °C. We correlated the duration with the environmental factors (ambient air temperature, water temperature and solar radiation) and the bees' body temperatures (thorax, head or abdomen). Results revealed that the duration of the foraging stays correlated best with the bees' head temperature. From the amount of crop loading and the duration of the foraging stays we estimated the mean suction rate per stay (crop loading/duration of foraging stay). It increased exponentially with T_a (Q_{10} = 1.8) and T_{head} (Q_{10} = 3.7). This steeper dependence on the head temperature elevated suction rate from v = 0.6 mg s^{-1} at T_{head} = 26 °C to v = 2.2 mg s^{-1} at T_{head} = 36 °C. Even at low T_a the bees regulated the thorax temperature at a high level during the whole foraging stay. Their high thorax temperature allowed them to regulate the head temperature high enough (>22 °C) to guarantee a proper function of the suction pump, and this way to shorten the duration of foraging trips. This optimizes foraging efficiency.
Supported by the Austrian Science Fund (FWF; P 16584-B06, P 20802-B16).

11.P2. Amino acid content and nectar preference in forager honeybees

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Dual choice feeding tests were performed to verify a preference of forager honeybees for artificial nectars supplemented with various amino acids. Proline-containing nectar was preferred over a solution containing only sugars. Daily individual consumption was initially higher also for alanine-containing nectar, but the difference became not statistically significant afterwards. On the contrary, a negative response was found for serine. When the test was carried out with two nectars enriched with different amino acids, the same preference hierarchy was evident.

12.P1. Melissopalynological analysis of royal jelly from Crete, Greece

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Royal jelly, secreted from the hypopharyngeal and mandibular glands of nurse bees (*Apis mellifera* L.), is an important apicultural product which can offer a significant financial profit to beekeepers. As most food, the determination of geographical origin is important for the marketing of royal jelly. Melissopalynological analysis is commonly used to determine the geographical origin of apicultural products. The aim of this research was to study the pollen spectrum of royal jelly produced in the island of Crete in Greece. Pollen analysis of 23 royal jelly samples collected from several areas of Crete was carried out during Spring 2009. More than 40 pollen types were recorded in total. The majority of the pollen types found in the royal jelly samples was also located at the pollen flora around the apiaries, indicating that the determination of the geographical origin of royal jelly is possible. The most frequent pollen types observed in the samples were *Olea europaea,* Brassicaceae, Hypericum Type*, Eucalyptus* and Carduus Type.

12.P2. The impact of uncrystallizing process to the quality of the honey

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Practically all honey will crystallize sooner or later but very few unifloral honeys stays liquid for months or years (*Acasia, Robinia, Epilobium, Myrtaceae*) Only *Epilobium* honey in Finland has been found to stay liquid for longer time. Although in average 58 % of the products are liquid in retail trade in Finland.
We tested a small scale processing method to produce liquid honey from 19 crystallized honey lots. The pollen analysis and basic laboratory analysis (moisture, main sugars, electrical conductivity, HMF, invertase activity) were measured. The two stage treatment included first prewarming the lot to 40 C and then immediately rapid flow warming to 69 C to remove the crystals. The samples were taken after both treatments and the development of HMF and invertase activity were followed. Also the crystallising process of the treated honey samples were followed for 120 days.
Melting honey in 40 C increased the HMF level of honey, but did not decrease the invertase activity considerably. Removing the crystals in short term (15 min) warming to 69 C did not increase the amount of HMF but decreased the invertase activity up to 60 % of the original level. Botanical origin and thereby the chemical properties of the processed honey lots are important to make successful processing. Our tests show, that it is possible to process honey also in small scale to make it stay liquid with out loosing considerably the chemical quality.

12.P3. Microbiological profile in fresh frozen organic bee pollen after thawing

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Bee pollen is a bee product of great interest for scientific, medical and economic point of view, it is important also for its psychological implications that evokes both organic food and natural way of life. Growing interest on commercial beepollen for human feeding incentives to investigate about its quality and food safety.
Amount of total mesophylic bacteria (TMB), yeasts and moulds has been investigated in fresh frozen commercial organic bee pollen after 24 and 48 hours since thawing at room temperatures. The experiment has been carried out by using 10 grams of polyfloral bee pollen in 90 ml of sterile physiological solution in three replicates. TMB, yeasts and moulds have been detected in all samples and in both room temperature exposure times. TMB ranged between $8 \cdot 10^4$ to $3.4 \cdot 10^5$ CFU/ml respectively at 24 and 48 hours. Yeasts and moulds ranged between $2.4 \cdot 10^3$ to $3.4 \cdot 10^5$ CFU/ml respectively at 24 and 48 hours. Results indicate that at least in these experimental conditions bee pollen is a quite stable bee product.
Deeper investigations need to measure and establish the biotic and not-biotic parameters that drive organic bee pollen's
shelf-life.

12.P4. Sensory analysis of honeys with Ericaceae nectars

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Sensory assessment is one of the most important analysis to determine the quality of honey. The organoleptic profile of any food commodity is useful to know the consumers' acceptance. In Spain, Ericaceae honeys are very appreciated for their strong colours and flavours. Using a panel of five assessors, we have developed an organoleptic analysis on 20 samples of honeys from Burgos (Spain) most heather unifloral and some of them multifloral, but with an important contribution of Ericaceae. The parameters determined were colour, shade, persistence and intensity of flavour (odour and taste), odour description, sweetness, acidity, bitterness, salty taste, aroma description after tasting, astringency, refreshing, crystallisation rate and other characteristics. The colour of the vast majority of the samples was brown. Some honeys had reddish/orange tones, and one sample was ochre-coloured. In general, the intensity of aroma was strong, and its persistence long, with a floral odour with woody accents. Sweetness and acidity were classified as weak to medium, whereas bitterness was classified as medium to strong. The aftertaste of the honeys analyzed was fresh, and in some samples slightly spicy or binding flavoured. The consistency of samples was creamy.

12.P5. Occurrence of Yeasts in Raw Honey

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Microflora in honey is of great interest, however, few have been documented in Thailand, regarding to yeasts residing in raw honey. In this study, we isolated yeasts in raw honey (31 samples) of 11 different bee species. Total yeast counts were ranging from 50 to 3.7×10^4 CFU ml^{-1}, with the higher counts observed in samples of *Apis florea* and *Trigona laeviceps*. Two main groups of yeast isolates, classified as *Candida* and *Pichia*, were found to be associated with samples of both the honeybees and the stingless bees. *Zygosaccharomyces* sp. and *Starmerella* sp. were occasionally present in some raw honey samples. *Debaryomyces* sp., *Kodamaea* sp., *Rhodotorula* sp. and *Schizosaccharomyces* sp. were also found. The members of the genus *Zygosaccharomyces* are generally known for their ability of fermenting hexose sugars such as glucose and fructose, and significantly resist to high osmotic pressure. These characteristics might cause the spoilage of honey. Therefore, good manufacturing practice should be concerned to avoid the proliferation of such yeasts in honey.

12.P6. The aroma profile of thyme honey

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Thyme honey has bright amber color and high fluidity and it is famous for its special aroma and flavor. Aroma profiles are useful for the characterization of the honey and the identification of the botanical source through typical volatile marker compounds and for the assessment of the authenticity and quality. To describe the volatile fingerprinting of thyme honey we collected samples from collaborates beekeepers from the island of Cyprus and we chose those that corresponded to the typical thymus honey according to their organoleptic, microscopic and physicochemical characteristics. We rejected samples that have pollen content of thymus less than 20%, diastase activity less than 19 DN and hydroxymethylfurfural (HMF) more than 14 mg/Kg to be certain that the samples were fresh, unheated and authentic.
For the extraction of the volatile compounds the purge and trap thermal desorption technique was used. The separation and detection of the isolated compounds carried out using a as Chromatograph – Mass Spectrometer system.
A total of 139 volatile substances were detected. The main volatile compounds were: phenylacetaldehyde, benzaldehyde, decanal, octane, benzyl-acetonitrile, 3-methyl-butanal, 1-pentanol, and 2-methyl-1-butanol. The relative concentrations for the two dominant compounds, phenylacetaldehyde and benzaldehyde ranged from to 70,7 to 1.640,4 μg Kg^{-1}, and from 76,6 to 218,8 μg Kg^{-1}respectively. Twenty four compounds were detected in all samples and the combination of these substances could be used for the determination of botanic origin of thyme honey.

12.P7. How Spanish perceive Bolivian pot honeys from six Meliponini species

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The project on meliponiculture in Bolivia promotes stingless beekeeping and agroforestry for sustainable development. A great effort is promoted by the Association of Native Honey Producers (Apromin), with forty families from the Amboró National Park who received financial support from PNUD for a honey house in Santa Fe. Packaging found a creative solution based on traditional spheroidal pottery named *puño*, which simulates darkness inside the hive. Each associate started with one hive and increased up to 15 to 40. Stingless bee honey yield is about 1-15 kg/year, but the fact that their honey is highly appreciated for the medicinal properties, increases the price up to 10-25 times the cost of *Apis mellifera* honey. In this work honey pots were pierced and the honey produced by six species of meliponines was extracted by suction. These species will receive a further entomological identification, but local names, familiar to consumers are used for marketing purposes: 1. Suro negro. 2. Ereureú choco. 3. Obobosi. 4. Señorita. 5. Erereú barcino. 6. Suro choco. Besides the harmonized physicochemical and palynological standards, we measured the acceptance of pot honey from Bolivia by a Spanish panel from 20 students and staff at the University of Burgos in Spain, who never tasted it before. Selection of the panel required to be a honey user, and adequate physiological conditions. Sensory evaluation for consumer acceptance was done using a 10-cm line anchored with the words low and high. The six honey samples were presented at the same time in an individual booth of the sensory room with daylight. Water and toast were provided to clean the palate between samples. Instructions suggested to taste all honeys first from left to right, and then to rank each one in a free order, and describe a short reason of this choice. This procedure provided a baseline rating the following averages ± SD: Suro negro 5.6 ± 2.2, ereureú choco 5.0 ±2.5, obobosi 5.5 ±2.5, señorita 4.8 ± 2.4, erereú barcino 3.7 ± 2.1, suro choco 4.9 ± 2.2. Erereú barcino honey was very light amber similar to acacia honey, with a mild taste but was the honey with lowest score, followed by señorita and suro choco, due to sour taste, fermented and animal notes. This average acceptance could be improved by better knowledge of the honeys, and would be very interesting to compare it with acceptance of consumers from urban and countryside Bolivia.

12.P8. Characterization of honey sources by *Geographical Information System*

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The characterization of honey sources is a valuable tool to use more rationally the potential of the area and to improve the honey production in terms of quantity and quality.
Under the Italian research project "Integration of bee knowledge through the development and calibration of a model for the simulation of the beehive" a methodology based on technologies *Geographical Information System* (GIS) for identifying and georeferencing the bee plants on the area around the experimental apiary of Di.Va.P.R.A. situated in Grugliasco (Turin) was developed.
The procedure has the following phases:
- retrieval of the Carta Tecnica Regionale (CTR) 1:10.000, Regione Piemonte;
- delimitation of the study area by defining a circumference of 1.5 km radius centered on the experimental apiary (optimal bee flight radius);

- transformation of the reference system of the original CTR (Gauss-Boaga) in UTM 32N WGS84;
- cartographic editing of images from Google Earth to update the conditions of the local anthropic and plant context;
- updating the contents of the CTR by photo interpretation of GoogleEarth 2007 images;
- recognition, vectorization and attribute assignation of the entities of interest recognized with GoogleEarth georeferenced imagery and verified in the field;
- derivation of honey potential by calculations made on the attribute fields of vector maps produced, involving bibliographic data and geometric data derivable with GIS procedures;
- data analysis and summary statement.

This procedure allowed to detect the actual use of the study area and to generate the corresponding thematic map of potential honey production.

12.P9. Detection of Sulfa drugs in honey by Sulfa-Sensor honey and Charm II Sulfa test Honey: A Comparison

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Sulfa-Sensor Honey (Unisensor s.a., Wandre, BE) is a new generic monoclonal antibody test for the detection of sulfonamides in honey in 20 minutes. A 5-minute sample pretreatment is needed to release the sulfonamides that are chemically bound to the sugars. The colour development on the dipstick is evaluated by a ReadSensor. Charm II Sulfa Drug Test for Honey (Charm Sciences Inc., Lawrence, MA) is a microbial receptor assay. A long hydrolysis and extract clean-up procedure is required to free sulfa drugs bound to sugars in honey and to eliminate interference from sulfa drug analogs such as para-aminobenzoic acid. In this assay there is competition between [H^3]-labeled sulfamethazine and sulfa drugs in the sample to bind to receptors on the microbial cells. The amount of [H^3] bound to the cells is measured with a liquid scintillation counter. The lower the cpm, the higher the amount of sulfa drug contamination of the sample.

The presence of sulfa drugs was screened with both screening tests in 71 honey samples that were bought on the market in different EU countries. Samples screened as positive were sent to an external lab for physicochemical confirmation by LC/MS-MS. Fifty-nine samples tested negative and 1 sample (112 µg/kg sulfathiazole) tested positive with both screening tests. False negative results: 1 sample with Sulfa-Sensor Honey (4 µg/kg sulfadimethoxine) and 2 samples with Charm II Sulfa Drug Test for Honey (35 and 25 µg/kg sulfathiazole). False positive results: 6 samples with Sulfa-Sensor Honey and 1 sample with Charm II Sulfa Drug Test for Honey.

12.P10. Presence of Antibiotics and Chemothera- Peutics in honey on the European market: situation in 2009

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In 2009 in 5 European countries (Belgium, Italy, Portugal, Spain and Switzerland) commercial honey samples were bought by different consumer's organizations and screened for residues of streptomycins, tetracyclines, macrolides, sulfonamides, fluoroquinolones, chloramphenicol and nitrofurans (except for Switzerland) by means of receptor and immunological tests. Samples screened as positive were sent to an external lab for physicochemical confirmation by LC/MS-MS.

In 11.6 % of the honey samples at least one residue (sulfathiazole, sulfadimethoxine, ciprofloxacin, norfloxacin, sulfadimethoxine, streptomycin and/or tylosin) was present. All honey samples were free from tetracyclines, chloramphenicol and nitrofurans at the detection level of

10, 0.2 and 1 µg/kg, respectively. For the 5 countries involved in the study, the following respective percentages of positive samples were obtained: 9.5% (BE, 2 out of 21), 13.3% (IT, 2 out of 15), 10.0% (PT, 2 out of 20), 20% (ES, 3 out of 15) and 6.7% (CH, 1 out of 15).
All 17 royal jelly samples bought in Belgium, Italy or Spain were free from residues of chloramphenicol and nitrofurans at the level of 0.2 and 1 µg/kg, respectively. There was no sampling of royal jelly in Portugal and Switzerland.

12.P11. Antiradical activity of honey bee-collected pollen of different botanical origin.

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The pollen collected by bees can be used as nutritive supplement, for embracing nutritional substances like carbohydrates, proteins, aminoacids, lipids, vitamins, and minerals and small quantity of micronutrients.
This beehive product also has several useful pharmacological properties, such as antibiotic, antioneoplastic, antidiarrhoeatic and as an antioxidant agent. The antioxidant activity of honeybee-collected pollen has been recognized as a free radical scavenger and as a lipid peroxidation inhibitor. This activity has been associated with the phenolic pollen content.
The purpose of the present study was to evaluate the effectiveness of extracts from honeybee-collected pollen as free radical scavengers.
Four extracts of different samples of pollen were made. Fourteen grams of honeybee-collected pollen were individually extracted three times in 200 ml ethanol-water solution (70% v/v) with a 30 min. maceration. All the supernatants of each type were brought together and formed the total extracts. These total extracts were evaporated to dryness.
The scavenging activity of extracts were determined through the use of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The total extracts of honeybee-collected pollen mixture and of the three different pollen samples showed to be effective antioxidants as free radical scavengers. Antiradical activity as determined by the DPPH radical scavenging method decreased in order: *Cistus Ladanifer* > *Castanea sativa* > *Erica* sp. > Mixture. In present investigations, great variability regarding the antiradical activity of the pollens was found, and all of them showed lower levels of antiradical activity than ascorbate.

Theme IV. BEEKEEPING AND BEE RESEARCH Symposium 13. Beekeeping and bee research in Turkey

13.P1. Differences in sperm traits of drones from queenright and laying worker colonies

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Some sperm traits of large drones from queenright colonies (QRC) were compared with those of small drones from laying worker colonies (LWC). The mean volume of ejaculate of drones from QRC (1.01 µl) was 53 % larger than that of drones from LWC (0.66 µl). The mean sperm number in drones from QRC (7.975 X 10^6) was significantly higher ($P < 0.001$) than that of drones from LWC (5.738 X10^6). Nevertheless, sperm concentration ($P < 0.001$) of drones from LWC (8.726 X 10^6 / µl) was significantly higher than that of drones from QRC (7.914 X 10^6 / µl). The drones from LWC produced less semen with higher sperm concentration compared to the drones from QRC.

13.P2. The viability of sperm in reproductive organs of instrumentally inseminated and naturally mated honey bee queens

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Instrumentally inseminated queens (IIQ) were compared with naturally mated ones (NMQ) with respect to sperm viability to detect whether instrumental insemination procedure has any negative effect on sperm viability in reproductive organs of IIQ. The viability of sperm in reproductive organs of IIQ and NMQ was determined at 4 h after they were inseminated or they mated. No significant difference was found between IIQ (88.6 %) and NMQ (87.6 %) in viability of sperm in lateral oviducts at 4 h. There was also no significant difference between IIQ (98.3 %) and NMQ (97.8 %) in viability of sperm in spermathecae at 4 h. The results showed that the insemination procedure had no negative effect on viability of sperm in reproductive organs of queens.

13.P3. Shape and genetic analysis on honey bees of Turkey

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The shape and genetic variability of six honey bee populations from Turkey (Bozcada, İzmir, Muğla, Kırklareli, Artvin and Hatay) were studied with geometric morphometry and five microsatellite loci. In studied honey bee populations, 11, 8, 15, 7, and 20 alleles were observed at Ap223, Ap019, Ap001, Ap243, and Ap289, respectively. The average number of alleles per locus per population varied between 3.8 and 9. Average observed heterozygosity values ranged between 0.386 (Muğla) and 0.550 (Kırklareli). The gene diversity values for populations studied varied between 0.351 and 0.608. These results suggest that there is a considerable genetic differentiation in Turkish honey bee populations. CVA based on geometric morphometrics data also differentiates the populations quite well showing the shape differences among populations and three main groups are formed. Deformation grids of the landmarks demonstrate the shape differences in the wings of honey bees. Homologous points

(Landmarks) in the wings indicate an elongation of the wing in Mugla honey bee population whereas deformation grid pattern suggests some shrinking in the wings of Hatay honey bee population. Artvin honey bee population shows some expansion in the mid region of the wing. UPGMA cluster analysis from geometric morphometric data illustrated that Artvin honey bee population was seperated from all other populations.

Theme V. OPEN SESSIONS Symposium 15a. Open session

15.P1. Quality of semen of mature drones kept in different temperature

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The aim of the study was to evaluate the number of live spermatozoa in drone semen and in spermatheca of queens inseminated with semen of drones kept in different temperature (conditions) before collecting semen for insemination.
Three groups of Carniolan sister queens, "Marynka" line (30 individuals in each group) were inseminated at the age of 6-7 days with two doses of semen (2 x 4 ml) collected from drones kept at temperature of 10°C, 35°C and 40°C. The number and viability of spermatozoa in the semen collected from drones and in queens' spermatheca 48 hours after insemination were checked. It was found that in volume of 1µl of semen collected from few drones (regardless given temperature) was about 10 million of spermatozoa. The lower percentage of live spermatozoa was noticed in the semen of drones kept at the temperature of 10°C and 40°C (respectively 64.8% and 71.5%) than in those kept at optimum temperature of 35°C (90.2%).
The number of spermatozoa in spermatheca of queens inseminated with semen collected from drones kept at 35°C was significantly higher (7.011 million) than in spermatheca of queens inseminated with semen drones kept in low and high temperature (respectively 6.200mln and 5.900mln). Significantly lower number of live spermatozoa was found in spermatheca of queens inseminated with semen of drones that were kept at 40°C (72.3%). The highest mortality and some semen residue in queens' oviducts after 48 hours from insemination were found in the group of queens inseminated with semen of drones kept at low temperature (respectively 6.5% and 10%).

15.P2. Effect of mixtures of carbon dioxide (CO2) with nitrogen (N2) on initial oviposition of instrumental inseminated honeybee queens

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During instrumental insemination, honey bee (*Apis mellifera*) queens are usually anaesthetized with carbon dioxide. This gas is also used to accelerate oviposition of instrumentally inseminated queens. High CO_2 concentrations have been found to be harmful to the honey bees. Here we assess the usefulness of CO_2/N_2 mixtures for anaesthetising queen bees to be inseminated instrumentally.
Carniolan queens (*A. m. carnica*) were instrumentally inseminated on the 7th day of life with 8 µl semen collected from drones of the same subspecies. During instrumental insemination the queens were treated with different gases. Queens of the first group (n = 34) were anaesthetised with a mixture of 40% CO_2 and 60% N_2, those from second group (n = 33) with 60% CO_2 and 40% N_2, and those from third group (control group, n = 31) with 100% CO_2. Two

days after insemination, queens were additionally treated with their respective gas mixtures for 3 minutes. The treatments and observations were made in two replicates.
The queens treated with 60% CO_2 and 40% N_2 and 100% CO_2 started oviposition on average after 7.2 ±4.0 and 8.1 ±4.2 days, respectively, i.e. significantly earlier from those anaesthetised with a mixture of 40% CO_2 and 60% N_2 which started oviposition on average 10.3 ± 4.1 days after their insemination. The gas composition did not affect the percentages of queens starting oviposition, which were 85.3% for 40% CO_2 and 60% N_2, 87.9% for 60% CO_2 and 40% N_2, and 83.9% for 100% CO_2.

15.P3. APIPOP: "Cells Availability" module

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Under the project PRIN 2007 entitled "Integration of bee knowledge through the development and calibration of a model for the simulation of the hive" is used ApiPop computer model, developed with the modelling environment SEMoLa (Simple, Easy to use, MOdelling Language). This is a tool for development and simulation of continuous dynamical systems (flows) or discrete (events), deterministic or stochastic, the objective is to simulate the complex interactions that occur in the hive.
Among the different modules that compose the model, that of the availability of cells is responsible for identifying all the factors (variables, constants, etc..) influencing it, identifying both the quantity of cells in a hive and their availability for the different needs of the colony (brood rearing, storage of honey and pollen).
In order to calibrate the model, tests were conducted in spring - summer of the years 2008 - 2010.
From the data acquired in the test hives variations in the number of cells for the brood (female and male) and the number of cells occupied by stocks (honey and pollen) could be determined within each year. The results, which are dependent by the strength of the colony, the health of bees, the use of food resources to the season and climate, were used for the implementation of the theoretical model.

15.P4. Morphological traits of bees kept in Poland and their changes over the past 20 years

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Bee races the most commonly kept in Poland are *Apis mellifera carnica*, *A. m. Caucas ica* and *A. m. mellifera*. Only the Black bees (*A. m. mellifera)* and several breeding lines of Carniolan bees are local, remaining ones are imported. Mathematical models for discrimination of bee races were developed in the early 80's. These models were created for each race individually based on the length of the proboscis, the width and IV tergit and the cubital index. Bees to be regarded as pure should have all these features at the certain level.
Material for the models construction was 832 samples of bees elaborated in the 70'. Since the models have been introduced into the practice the majority of breeding apiaries inseminated bee queens to maintain pure race colonies. Only a few apiaries had then drone areas. Mean values of the morphological features, used for construction the models were:
-length of proboscis (mm): *A. m. carnica*- 6.458, *A. m. caucasica*- 6.976, *A. m. mellifera*- 6.115
-width of IV tergit (mm): *A. m. carnica*- 2.302, *A. m. caucasica*- 2.242, *A. m. mellifera*- 2.356

-cubital index (in Alpatov notation[%]): *A. m. carnica*- 51.2, *A. m. caucasica*- 54.7, *A. m. mellifera*- 61.4
After nearly 20 years bees from 1042 colonies of breeding apiaries were examined. The average length of the proboscis was reduced in the Caucasian bees (-3%) while in the Black bees (2.2%) and Carniolan proboscis became slightly longer (0.8%). The average width of the IV tergit increased in Caucasian bees (0.7%) while decreased in Cariolan (-0.8%) and Black bees (-2.3%). The highest change in the cubital index was for Caucasian (-5.8%) and for Black bees (-10.6%). It is thought that the changes were caused by import of various morphologically bees or an accidental crossing of different bee breeds.

15.P5. Shortage of authorized veterinary medicines for varroatosis control

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The availability of appropriate veterinary medicinal products to control Varroa mite infestation of honey bees has become a primary need for beekeeping. In fact, according to EU regulation governing the use of veterinary medicinal products only authorized drugs can be applied and their active ingredients need to be evaluated for Maximum Residues Limit (MRL) (Regulation EU N. 35/2010). After the onset of resistant mites against pyretroids and to a lesser extent to amitraz and cumaphos, the attention has been drawn to active ingredients that could guarantee an adequate control of varroatosis. Fluvalinate-, amitraz-, cumaphos-, formic and oxalic acid-, as well as thymol-based veterinary medicines are now available in Europe according to national registration and centralized procedure as well as to mutual recognition procedure. Unfortunately, not all the above mentioned veterinary medicines are available in all EU countries with many differences emerging in terms of registration procedure and approaches by the competent regulatory authorities. Thymol-, pyretroids- and amitraz-based medicines are available in several countries, according to previous registration and despite the well known resistance developed by Varroa mites against pyretroids and amitraz. Organic acid-based medicines i.e. oxalic and formic acid are instead available only in a limited number of countries following national registration. According to the emergency situation occurred in Italy due to the limited effectiveness of thymol-based medicines and the high level of pharmacoresistance to pyretroids, a registration procedure was initiated to make oxalic acid available for varroatosis control in brood less time. It is considered necessary that the same procedure could be made available also for formic acid-based medicines in order to improve the set of veterinary medicines to be use against varroatosis in brood right time.

15.P6. Survival of untreated honeybee colonies under high infestation with *Varroa destructor*

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Pre-selected honeybee colonies from private beekeepers participating in a breeding project were overwintered without treatment against varroosis in different apiaries in Germany. Colony development, infestation levels with *Varroa destructor*, viral infections and nosema infestations were observed during fall 2009 and correlated with colony survival in the following winter. Colonies which overwintered successfully without treatment were stronger and showed lower infestation levels with *V. destructor* than colonies which did not survive without treatment. Both groups of colonies had similar level of DWV, ABPV and nosema infections but they showed different level of CBPV infection. Our results clearly point out the possibility to overwinter bee colonies without varroa treatment, if they are monitored continuously in late summer and fall. Such

viability tests may be used as a selection criterion by breeders and allow the establishment of threshold values as a guide line for beekeepers to make a decision about when to treat a colony against varroosis.

15.P7. The Visitor - *Potosia opaca* Fabricius comes back in Italian apiaries

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Big beetles (2 cm x 1,5 cm) were detected in honeybee hives of an apiary settled in Regional San Rossore Migliarino and Massaciuccoli Natural Park, Pisa, Italy. Specimens, opaque black, on the combs eating honey and stored pollen. These beetle is *Potosia opaca* Fabricius (Coleoptera, Cetonideae). Four beetles were found in a hive, two in a second hive and one in other two hives, all the hives were without grid-doors. All the other hives of the apiary were with the grid-doors. This insect is a flower beetle, feeding on nectar and pollen, and developing as pre-imagine form in rooted wood or other organic matter. These beetles were not attacked by bees. Moreover, if disturbed or tickled by hand, the beetles showed the typical behavior of thanatosis. In the past *Potosia opaca* was associated to honeybees as a cleptoparasite that steal honey. Nowadays is almost not mentioned in modern beekeeping handbook , because it became rare probably for rural pollution and lack of not disturbed or wild places for its reproduction. The presence of *Potosia opaca* Fabricius is not dangerous for honeybees and hive products, and would be interesting evaluate these beetle as an indicator of the area's environmental quality.

15.P8. Brood comb and humidity regulation in honeybee nests

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Silk cocoons are spun by honeybee (*Apis mellifera*) larvae and subsequently incorporated into the cell walls of the wax combs in which they develop. The accumulation of this hygroscopic silk in the thousands of cells used for brood rearing may significantly affect nest homeostasis by buffering humidity fluctuations. Our study investigated the extent to which the comb may influence homeostasis by quantifying the hygroscopic capacity of the cocoons spun by honeybee larvae. When comb containing cocoons was placed at high humidity, it absorbed 11% of its own mass in water within four days. Newly drawn comb composed of hydrophobic wax and devoid of cocoons absorbed only 3% of its own mass. Therefore, the accumulation of the cocoons within the combs may increase brood survivorship by maintaining a high and stable humidity in the cells.

15.P9. The value of some new melliferous species as a bee forage plants in Poland.

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A good forage plants are the base of modern beekeeping. They are very important not only for harvest of honey in apiary, but the primarily for proper and sustainable development of honeybee colonies. Therefore, the progressive estimation of beekeeping value of new

melliferous plants which are interesting for honey bees were conducted in Institute of Pomology and Floriculture, Division of Apiculture in Pulawy.
The experiments were established on podsolic soil in Collection of Honey Plants. Such soils were very often excluded from cultivation and could be used for improving of bee forage. During the 3 years of study, biology of blooming and foraging flowers by insects were observed. Moreover, abundance of blooming and abundance of nectar secretion by flowers were measured. On the base of measurements the sugars efficiency (output) of particular species per acreage unit were estimated, what is the main attribute of beekeeping value of plant.
In recent years some species, new as a bee forage plants in Poland, were tested. Three of them proved promising as a propositions for improving of forage flow for bees. There were in blooming order: *Centaurea macrocephala* Puschk. ex Willd., *Ligularia dentata* (A. Gray) H. Hara. and *Caryopteris incana* (Thunb. ex Houtt.) Miq. The blooming period of the last two species lasted from August to half of September and from September to half of October, respectively, when the shortage of forage flow for bees in Poland is observed. The sugars output was 293, 136 and 106 kg/ha for *Centaurea, Ligularia* and *Caryopteris,* respectively.

15.P10. UMT Prade: A new French partnership to ensure the transfer of scientific knowledge to the protection of bees

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UMT PrADE is a French unit which associates different groups of stakeholders in the bee protection, from the researcher to the apicultural engineer. We establish a source of validated and recognized information allowing to improve the knowledge on the decline of bees and to conceive technical solutions capable of struggle it. This conception of technical solutions aims at the development of operational tools for the help to the reasoning of the protection of bees.
The scientific results should be assimilated by the users in term of management of the agricultural and apicultural systems. Concerning the finality of our works, we direct it on the evolution, even the construction, of decision-making tools to the agricultural practice, the landscape management or the management of the livestock of beekeepers. It is thus a question i) of co-building valuation methods and tools of piloting to enhance reliability of the knowledge on the decline of bees and more generally on the evolution of their populations, ii) to ensure a global transfer of results to agricultural and apicultural practices.
So, we try to answer three series of determining objectives: improvement of diagnostics, innovation in the agro-ecological measures, study of the factors of pressure. In each of these three domains, works are carried out at various levels of biological organization. A global approach is privileged allowing to reason all the constituents involved in the health of populations (agricultural practices, landscape, resources, pathogenic agents, climate), to take into account the variety of situations and estimate them in a global way.

15.P11. Electrophoretic and Enzymatic Investigations at Different Developmental Stages of Honey Bees (*Apis mellifera* L.)

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Three subspecies of *Apis mellifera* L. (*A. m. caucasica, A. m. carnica and A. m. syriaca*) from different climatic regions were evaluated electrophoretically at different developmental stages by means of four enzymes, namely Phosphoglucomutase (PGM), Hexokinase (HK), Phosphoglucose isomerase (PGI) and Glucose-6-phosphate dehydrogenase (G6PD). It is determined that only *Pgm* and *Hk* loci were polymorphic. Allele and genotype frequencies at *Pgm* locus changes seasonally whereas *Hk* locus does not exhibit seasonal variation. In previous studies, seasonal fluctuation of *Pgm* genotype frequencies among adult bees Here we investigated at which developmental stage shifting to heterozygotes prior to winter occurs.
It is found that there is a seasonal fluctuation throughout the year in *Pgm* genotype frequencies at each developmental stage studied as observed at adult stage and correlated with enzyme activity and glycogen content. Results of this study provided further information about the relationship between carbonhydrate metabolism and enzyme polymorphism of honey bees.